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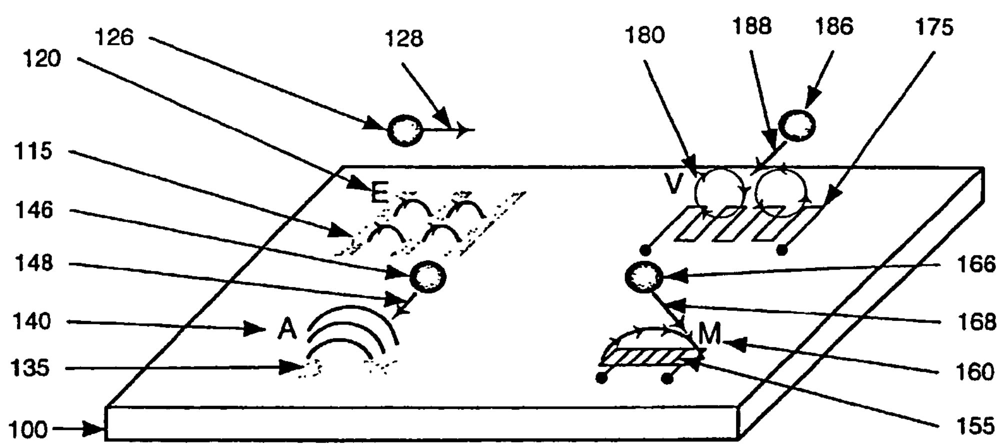

(57) Abstract: This invention relates generally to the field of moiety or molecule manipulation, e.g., in a chip format. In particular, the invention provides a chip for generating fields, which chip comprises: a) a substrate; and b) at least two different types of structures located on said substrate, wherein each of said structures is capable of, in combination with an external energy source generating one type of physical field. Combinations and apparatuses containing single or multiple chips, and methods using the chips for manipulating a moiety or molecule are also provided.

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APPARATUSES CONTAINING MULTIPLE FORCE GENERATING ELEMENTS AND USES THEREOF

Technical Field

This invention relates generally to the field of moiety or molecule manipulation in a chip format. In particular, the invention provides a chip for generating physical fields, which chip comprises: a) a substrate; and b) at least two different types of built-in structures on said substrate, wherein each of said structures is capable of, in combination with an external energy source, generating one type of physical field. Physical field has such characteristics that when a moiety having appropriate properties is introduced into the field, forces are produced on the moiety as the result of interaction between the moiety and the field. Physical fields include electric, magnetic, acoustic, optical, velocity field and other fields. Combinations and apparatuses containing single or multiple chips, and methods using the chips or apparatuses for manipulating a moiety or molecule are also provided.

Background Art

There are generally two types of biochips. The first type, *i.e.*, the passive type, does not utilize externally applied physical forces to manipulate, control or influence molecules and particles for chemical, biochemical or biological reactions. The reaction process involves in thermal diffusion of molecules/particles and involves in naturally occurring forces such as earth gravity on any particles and earth-magnetic forces on any magnetic particles. The second type, *i.e.*, active type, utilizes externally applied physical forces to promote, enhance or facilitate desired biochemical reactions or processing and to decrease or reduce any undesired effects. A typical example of passive types is the DNA chips or DNA arrays (e.g. Lockhart D.J. and Winzeler E.A., Nature, Vol. 405, No. 6788, pages 827-836), where the hybridization between the probe oligo-nucleotide molecules or cDNA molecules immobilized on the chip and the target molecules in the solution over the DNA chip occurs only when the target molecules diffuse to in contact with the probe molecules. In contrast, a typical example for active biochips is the electronic chips, as disclosed in US

patent 6,017,696, "Methods for electronic stringency control for molecular biological analysis and diagnostics" and disclosed in the US patent 6,051,380, "Methods and procedures for molecular biological analysis and diagnostics". On these electronic chips, the charged DNA (or other) molecules are electrophoreticly directed or drawn to microelectrodes with opposite charge polarities, on which probe molecules have been immobilized.

Although the above examples deal with DNA molecules, the same classification, in terms of active versus passive biochips, applies to the biochips' processing other molecules or molecular complexes or bioparticles, *e.g.*, cells, bacteria, virus, proteins, nucleic acid molecules. In addition to molecule hybridization in the above examples, the biochemical reaction or processing on a chip includes many other steps such as molecule/particle transportation, molecule/particle separation, molecule/particle discrimination, molecule-molecule interaction and molecule-particle interaction. For example, separation of cancer cells from a mixture with normal cells on a dielectrophoresis (DEP) chip is considered an active biochip biochemical processing. Compared with passive chips, the advantages of the active biochip include fast reaction, potentially better sensitivity for detection, analysis or assay of low-concentration molecules or particles or bioparticles, and potentially better discrimination between different types of molecules and between different types of particles/bioparticles.

Use of combined forces for certain purposes has been reported. Yasuda et al. show that using competing electrostatic and acoustic radiation forces on particles placed in a standing acoustic wave and a DC electric field, spatial separation of polystyrene and aluminum beads of different size and charges within the fluidic chamber were achieved (Yasuda et al, *J. Acoust. Soc. Am.*, Vol. 99(4), pages: 1965-1970 (1996); and Yasuda et al., *Jpn. J. Appl. Phys.*, Vol. 35(1), pages: 3295-3299, (1996)). In this case, four discrete platinum wires were used for generating a uniform DC electric field and producing electrostatic forces on charged particles. Two piezoelectric transducers are positioned at certain distances from each other and are used to generate a standing acoustic wave. Such a system is of limited use for particle manipulation and separation since it involves in only a standing acoustic wave and a uniform DC electric field and is applicable to a limited number of particle types. The manipulation method and process are not flexible. The system can manipulate particles only in limited ways or directions. The system can not be

used to manipulate and handle particles for many different purposes, such as separating particles to obtain "purified" fractions.

In another report, optical radiation forces are used to position particles in a rotating electrical field (De Gasperis et al., *Meas. Sci. Technol.*, Vol. 9, pages: 518-529 (1998)). In this case, optical tweezers that result from the combination of an optical source and an optical circuit comprising lenses and other components are used exclusively for positioning particles or cells in a rotating electric field. The electrorotation field that is produced on an electrorotation chip is used for inducing particle rotation. Such a system is of limited use. In this system, particle rotations are used for characterizing particle properties, and are not used for manipulating the particles or cells. Optical radiation forces are used exclusively to control particle positions. The system can be used only for the purpose of characterizing particle dielectric properties. To obtain reliable particle property parameters from the electrorotation methods, particles should remain at fixed positions of the rotating electrical field so as to experience a constant rotating field strengths. The system cannot be used for many different purposes such as separating particle mixtures into different fractions.

In still another recent report, to achieve the purpose of positioning two particles in parallel, researchers employed an optical radiation force to control one particle and utilized a dielectrophoretic cage to position another particle (G. Fuhr and C. Reichle, in van den Berg et al. (Ed.), *Micro Total Analysis Systems*, 2000, 261-264; and T. Schnelle et al., *Appl. Phys.*, B Lasers & Optics, Vol. 70, pages: 267-274 (2000)). The optical radiation forces are effected through optical tweezers that result from the combination of an optical source and an optical circuit comprising lenses and other components. The dielectrophoretic cage is produced by using three-dimensional configuration of microelectrode elements on dielectrophoretic chips. Such a system is of limited use. It cannot be used for many different purposes, such as transporting particles on a chip.

The present invention addresses the limitations of previous chips or biochips. It is an objective of the present invention to provide a new class of chips, and the method of using these chips, for biochemical, biological, or chemical processing, reaction, assay or synthesis. It is another objective of the present invention to provide an active chip, especially an active biochip that can be generally applied to all chip areas, where biological, chemical, biochemical reactions and procedures can be performed through microfluidic processing and manipulation of molecules and particles on microfluidic devices and systems. It is still another objective of the present invention to provide a

generally applicable active biochip that is particularly applicable to the so-called lab-on-a-chip system, *i.e.*, the system that integrate many separate biochemical, biological or chemical tasks onto a single chip or multiple-interconnected-chips.

Disclosure of the Invention

This invention relates generally to the field of moiety or molecule manipulation in a chip format. In one aspect, the invention provides a chip for generating physical fields, which chip comprises: a) a substrate; and b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field.

In one embodiment, the built-in structures in the chip generate at least two different types of physical fields. The chip may comprise a plurality of structurally linked substrates. The chip may comprise two, three, four or more than four different types of built-in structures that are capable of generating two, three, four, or more than four different types of physical fields.

In one embodiment, the built-in structures in the chip are in the form of single units. The single units may be located in a portion of or in the entire chip. In another embodiment, the built-in structures in the chip comprise a plurality of microunits. Some or all the microunits may be individually addressable. Some or all of the microunits on the chip may be interconnected. The chip may further comprise means for selectively energizing any one of the plurality of microunits.

In one embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic, optical, and velocity fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic and optical fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic, and velocity fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, optical, and velocity fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of electric, acoustic,

optical, and velocity fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields selected from the group consisting of magnetic, acoustic, optical, and velocity fields. In another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields that do not include the combination of an optical radiation field and a non-uniform AC field. In still another embodiment, the built-in structures on the chip are capable of generating at least two different types of physical fields that do not include the combination of a standing-wave acoustic field and a uniform electrostatic field. In still another embodiment, the built-in structures on the chip are capable of generating physical fields that do not include the combination of electric fields and velocity fields.

In one embodiment, at least one built-in structure on the chip is capable of generating an electric field such as a uniform or non-uniform DC electric field, a non-uniform AC electric field that has a non-uniform distribution in field magnitude, or a non-uniform AC distribution in phase values of at least one field component. In one example, the built-in structure on the chip that generates electric field comprises at least one microelectrode element.

In another embodiment, at least one built-in structure on the chip is capable of generating a magnetic field. In one example, the built-in structure that generates a magnetic field comprises a ferromagnetic material. In another example, the built-in structure that generates a magnetic field comprises a microelectromagenetic unit.

In another embodiment, at least one built-in structure on the chip is capable of generating an acoustic field. In one example, the built-in structure that generates an acoustic field comprise a piezoelectric material.

In another embodiment, at least one built-in structure on the chip is capable of generating a velocity field. In one example, the built-in structure that generates a velocity [illegible]

array of electric-optical sources. In another example, the built-in structure on the chip that generates an optical field comprises a laser tweezers.

The built-in structures on the chip may be micro-scale structures. The micro-scale structures have characteristic dimension of basic structural elements in the range from about 0.1 micron to about 20 mm scale. The substrate in the chip may comprise various types of surfaces, such as a silicon, a silicon dioxide, a silicon nitride, a plastic, a glass, a ceramic, a rubber, and a polymer surface. The surface may be advantageously hydrophobic or hydrophilic, for different applications.

In another aspect, the invention provides a chip for generating physical fields, which chip essentially consists of a) a substrate; and b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field.

In another aspect, the invention provides a chip for generating physical fields, which chip consists of a) a substrate; and b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field.

In another aspect, the present invention is directed to a combination, which combination comprises: a) at least two chips comprising a substrate and at least two different types of structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field; and b) means for transporting a moiety to be manipulated between said chips.

In still another aspect, the present invention is directed to an apparatus, which apparatus comprises a chip comprising a substrate and at least two different types of structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field.

In another aspect, the present invention provides an apparatus for manipulating a moiety, which apparatus comprises: a) a substrate for holding or supporting a moiety to be manipulated; b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination of an external energy source, generating one type of physical force on said moiety. The internal structures of the apparatus are capable of generating at least two different types of physical forces on the moiety to be manipulated. The apparatus may comprise two, three, four, or more than four different types of internal structures and are capable of generating two, three, four or more

than four different types of physical forces on the moiety to be manipulated. In one embodiment, the internal structures are built-in structures located on said substrate. In another embodiment, the internal structures are not located on the substrate. The apparatus may comprise a plurality of structurally linked substrates.

In one embodiment, the internal structures of the apparatus are in the form of single units that are located in a portion of or in the entire substrate. In another embodiment, the internal structures of the apparatus comprise a plurality of microunits. Some or all the microunits may be individually addressable. Some or all the microunits may be interconnected. The apparatus may comprise means for selectively energizing any one of the plurality of microunits.

In one embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force, mechanical force, and optical radiation force. In another embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force, and mechanical force. In another embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force, and optical radiation force. In another embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, mechanical force, and optical radiation force. In another embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, acoustic force, mechanical force, and optical radiation force. In another embodiment, the internal structures of the apparatus are capable of generating at least two different types of physical forces selected from the group consisting of magnetic force, acoustic force, mechanical force, and optical radiation force. In another embodiment, the internal structures are capable of generating at least two different types of physical forces that do not include the combination of an optical radiation force and a conventional dielectrophoretic force. In another embodiment, the internal structures are capable of generating at least two different types of physical forces that do not include the combination of an acoustic force and an electrostatic force. In another embodiment, the internal structures are capable of generating at least two different

types of physical forces that do not include the combination of a mechanical force and an electric force.

In one embodiment, at least one internal structure of the apparatus is capable of generating an electric force. In one example, at least one internal structure is capable of generating an electrostatic force on charged particles. In another example, at least one internal structure is capable of generating a conventional dielectrophoretic force. In another example, at least one internal structure is capable of generating a traveling-wave dielectrophoretic force. The internal structure of the apparatus that generates electric field may comprise at least one microelectrode element.

In another embodiment, at least one internal structure of the apparatus is capable of generating a magnetic force. In one example, the internal structure of the apparatus that generates a magnetic field comprises a ferromagnetic material. In another example, the internal structure that generates a magnetic field comprises a microelectromagenetic unit.

In another embodiment, at least one internal structure of the apparatus is capable of generating an acoustic force. In one example, the internal structure of the apparatus that generates an acoustic force comprises a piezoelectric material.

In another embodiment, at least one internal structure of the apparatus is capable of generating a mechanical force. In one example, the internal structure of the apparatus that generates a mechanical force comprises at least one microfabricated tip/capillary. In another example, the internal structure of the apparatus that generates a mechanical force comprises an array of heating and/or cooling units. Such an array of heating and/or cooling units can produce thermal gradients and a velocity field in the medium that is placed in the vicinity of the array. The velocity field in the medium can then exert a mechanical force on a moiety that are placed in the medium.

In another embodiment, at least one internal structure of the apparatus is capable of generating optical radiation force. In one example, the internal structure on the apparatus that generates an optical force comprises a microfabricated array of optical lenses. In another example, the internal structures on the apparatus that generates an optical force comprises a microfabricated array of electric-optical sources. In another example, the internal structure of the apparatus that generates an optical force comprises a laser tweezers.

The internal structures on the apparatus may be micro-scale structures. The micro-scale structures have characteristic dimension of basic structural elements in the range from

about 0.1 micron to about 20 mm scale. The substrate in the apparatus may comprise various types of surfaces, such as a silicon, *e.g.*, a silicon dioxide, a silicon nitride, a plastic, a glass, a ceramic, a rubber, and a polymer surface. The substrate surface may be advantageously hydrophobic or hydrophilic, for different applications.

The apparatus may further contain a fluidic chamber that comprises a substrate and a housing for holding or supporting or containing moiety to be manipulated. The internal structures of the apparatus that are capable of producing at least two types of physical forces on the moiety to be manipulated may be located or not located on the substrate. The fluidic chamber may be a closed chamber that comprises at least one inlet port and at least one outlet port.

In one embodiment, the apparatus does not contain a monitoring or detecting device.

In another aspect, the present invention provides an apparatus for manipulating a moiety, which apparatus essentially consists of: a) a substrate for holding or supporting a moiety to be manipulated; b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination of an external energy source, generating one type of physical force on said moiety.

In still another aspect, the present invention provides an apparatus for manipulating a moiety, which apparatus consists of: a) a substrate for holding or supporting a moiety to be manipulated; b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination of an external energy source, generating one type of physical force on said moiety.

In yet another aspect, the present invention is directed to a combination, which combination comprises: a) at least two apparatuses each of which comprises a substrate for holding or supporting a moiety to be manipulated and at least two different types of structures internal to the apparatus, wherein each of said internal structures is capable of, in combination of an external energy source, generating one type of physical force on said moiety; and b) means for transporting a moiety to be manipulated between said apparatuses.

In yet another aspect, the present invention is directed to a method for manipulating a moiety, which method comprises: a) introducing a moiety to be manipulated onto a chip comprising a substrate and at least two different types of built-in structures located on said substrate, wherein each of built-in structures is capable of, in combination of an external

energy source, generating one type of physical field; and b) allowing the built-in structures of said chip, in combination of an external energy source, to exert at last two different types of physical forces on said moiety, whereby said moiety is manipulated by said physical forces.

In yet another aspect, the present invention is directed to a method for manipulating a moiety, which method comprises: a) introducing a moiety to be manipulated into an apparatus comprising a substrate for holding and supporting the moiety to be manipulated and at least two different types of structures internal to the apparatus, wherein each of said internal structures is capable of, in combination of an external energy source, generating one type of physical field; and b) allowing the internal structures of said apparatus, in combination of an external energy source, to exert at last two different types of physical forces on said moiety, whereby said moiety is manipulated by said physical forces.

In one embodiment, the method for manipulating moiety utilizes at least two different types of physical forces that are exerted on the moiety sequentially. In another embodiment, the method for manipulating moiety is utilizing at least two different types of physical forces that are exerted on the moiety simultaneously.

In certain embodiments, a plurality of moieties are manipulated simultaneously by utilizing the manipulation method described above. For example, a plurality of moieties are manipulated simultaneously through use of more than one type of physical forces so that at least two different moieties are manipulated by different types of physical forces. In some other embodiments, a plurality of moieties are manipulated sequentially. For example, a plurality of moieties are manipulated sequentially through use of more than one type of physical forces so that at least two different moieties are manipulated by different types of physical forces.

In other embodiments, the moiety to be manipulated is contained in a mixture and the moiety is selectively manipulated using the manipulation method described above. In yet other embodiments, the moiety to be manipulated constitutes a mixture and the entire mixture is manipulated.

The present manipulation method is applicable to any moiety type such as a cell, a cellular organelle, a virus, a molecule and an aggregate or complex thereof. The cell may be an animal cell, a plant cell, a fungus cell, a bacterium cell, a recombinant cell or a cultured cell. The cellular organelle may be a nuclei, a mitochondrion, a chloroplast, a ribosome, an ER, a Golgi apparatus, a lysosome, a proteasome, a secretory vesicle, a

vacuole or a microsome. The molecule may be an inorganic molecule, an organic molecule or a complex thereof. The inorganic molecule may be an ion such as a sodium, a potassium, a magnesium, a calcium, a chlorine, an iron, a copper, a zinc, a manganese, a cobalt, an iodine, a molybdenum, a vanadium, a nickel, a chromium, a fluorine, a silicon, a tin, a boron or an arsenic ion. The organic molecule may be an amino acid, a peptide, a protein, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a vitamin, a monosaccharide, an oligosaccharide, a carbohydrate, a lipid or a complex thereof.

The present manipulation method can be utilized in transporting, focusing, enriching, concentrating, aggregating, trapping, repulsing, levitating, separating, fractionating, isolating or directing linear or other directed motion of the moiety.

In yet another aspect, the present invention is directed to a method for manipulating a moiety, which method comprises exerting at least two different types of physical forces on a moiety, whereby said moiety is manipulated by said physical forces.

Brief Description of the Drawings

Figure 1. A schematic drawing of a multiple-force chip or an apparatus of the present invention, showing that the chip (or the apparatus) is capable of generating electric field, magnetic field, acoustic field and velocity field.

Figure 2. A schematic drawing of a multiple-force chip of the present invention, showing that the chip comprises a plurality of micro-units, each unit is capable of generating electric, magnetic and acoustic field. Each unit may further comprise biological elements.

Figure 3. Schematic drawing of examples of two-force chips of the present invention, capable of producing acoustic forces and conventional dielectrophoretic (DEP) forces.

A) Interdigitated microelectrodes for producing conventional-DEP forces are fabricated on a substrate. The substrate is made of piezoelectric materials and is capable of generating acoustic fields.

B) The plane electrodes covered on both surfaces of a piezoelectric transducer (the piezoelectric substrate) are used to energize piezoelectric materials for generating acoustic fields. Circular-type microelectrodes for producing conventional-DEP forces are fabricated on a solid substrate (the second substrate). The DEP substrate (i.e. the

second substrate) is bound to or attached to the piezoelectric substrate to form a two-force-chip.

C) An array of acoustic wave sources for producing acoustic fields is fabricated on a solid (first) substrate. An interdigitated electrode array for producing conventional-DEP forces is fabricated on a solid (second) substrate. The second substrate (for DEP force) is bound to or attached to the first substrate to form a two-force-chip.

Figure 4. Schematic drawing of a fluidic chamber that comprises a multiple-force chip at the chamber bottom.

Figure 5. Schematic drawing of examples of two-force chips of the present invention, capable of producing acoustic forces and magnetic forces.

A) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is fabricated on a substrate. The substrate is made of piezoelectric materials and is capable of generating acoustic fields.

B) An array of acoustic wave sources for producing acoustic fields is fabricated on a solid, piezoelectric substrate. A microelectromagnetic unit array is fabricated on a (second) substrate. The second substrate (for producing magnetic forces) is bound to or attached to the first piezoelectric substrate (for producing acoustic forces) to form a two-force-chip.

C) An array of microelectromagnetic units (or electromagnetic units) is co-fabricated with an array of acoustic transducers on a solid substrate.

Figure 6. Schematic drawing of examples of two-force chips of the present invention, capable of producing dielectrophoretic (DEP) forces (i.e., conventional DEP forces and/or traveling-wave DEP forces) and magnetic forces.

A) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. An array of interdigitated electrodes is then fabricated on the surface-furnished dielectric layer.

B) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. A four-phase, linear,

traveling-wave dielectrophoresis electrode array for producing traveling-wave DEP forces is then fabricated on the surface-furnished dielectric layer.

C) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. An array of diamond-shaped electrodes for producing conventional DEP forces is then fabricated on the surface-furnished dielectric layer.

D) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. A spiral electrode array for producing traveling-wave DEP and conventional DEP forces is then fabricated on the surface-furnished dielectric layer.

Figure 7. Schematic drawing of examples of two-force chips of the present invention, capable of producing electrophoretic forces and magnetic forces.

A) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. An array of individually addressable electrodes is then fabricated on the surface-furnished dielectric layer. Each electrode element is of rectangular shape.

B) An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is first fabricated on a substrate. The electromagnetic unit array is then coated with a dielectric layer that is polished for smoothness. A four-phase, parallel electrode array for producing traveling-wave electrophoresis forces is then fabricated on the surface-furnished dielectric layer.

Figure 8. Schematic drawing of examples of two-force chips of the present invention, capable of producing electrostatic (i.e., electrophoretic) forces and thermal-convection based mechanical forces. An array of electrically heating elements is fabricated on a substrate. The heating elements are then coated with a dielectric layer that is polished for smoothness. An array of individually addressable electrodes for producing electrostatic forces is then fabricated on the surface-furnished dielectric layer. The electrodes in the individually addressable electrode array are of hexagon shapes.

Figure 9. Schematic drawing of examples of two-force chips of the present invention, capable of producing dielectrophoretic forces and thermal-convection induced mechanical forces. An array of electrically heating elements is fabricated on a substrate. The heating elements are then coated with a dielectric layer that is polished for smoothness. An array of individually addressable electrodes for producing dielectrophoresis forces is then fabricated on the surface-furnished dielectric layer. The electrodes in the individually addressable electrode array are of circular shapes.

Figure 10. Schematic drawing of examples of three-force apparatuses of the present invention, capable of producing magnetic forces, traveling wave dielectrophoretic (DEP) forces and optical radiation forces. An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is fabricated on a substrate (the first substrate). The electromagnetic units are then coated with a dielectric layer that is polished for smoothness. A four-phase, parallel, traveling-wave dielectrophoresis electrode array is then fabricated on the surface-furnished dielectric layer. Thus, a two-force chip capable of magnetic forces and traveling-wave DEP forces is formed on the first substrate. The optical chip comprises an array of optical lenses fabricated on an appropriate substrate (second substrate). The two-force chip and the optical chip are bound together with a spacer in between. The spacer has a channel cut in the middle. Furthermore, an inlet port and an outlet port are fabricated on the optical chip.

Figure 11. Schematic drawing of examples of three-force apparatus of the present invention, capable of producing acoustic forces, magnetic forces, traveling wave dielectrophoretic (DEP) and/or conventional dielectrophoretic (DEP) forces.

A) The plane electrodes covered on both surfaces of a piezoelectric transducer (the piezoelectric substrate) are used to energize piezoelectric materials for producing an acoustic field. An array of microelectromagnetic units or electromagnetic units for producing magnetic forces is fabricated on a substrate (the second substrate). The electromagnetic units are coated with a dielectric layer that is polished for smoothness. A four-phase, parallel, traveling-wave dielectrophoresis electrode array capable of producing traveling wave DEP forces is then fabricated on the surface-furnished dielectric layer. The second substrate that supports both electromagnetic unit array and

traveling-wave DEP electrode array is bound to or attached to the piezoelectric substrate to form a three-force chip.

B) A substrate made of piezoelectric materials is used to produce acoustic field. The plane electrodes are used to cover both surfaces of the piezoelectric substrate. A surface of the piezoelectric substrate is then coated with a dielectric layer (the first dielectric layer) that is polished for smoothness. An array of microelectromagnetic units or electromagnetic units is fabricated onto the (first) dielectric layer. The electromagnetic units are then coated with a dielectric layer (the second dielectric layer) that is polished for smoothness. An array of concentric circle electrodes capable of producing conventional DEP and traveling-wave DEP forces then fabricated on the surface-polished dielectric layer (the second dielectric layer).

C) An array of acoustic-wave sources for producing acoustic fields is fabricated on a solid substrate (the first substrate). An array of microelectromagnetic units or electromagnetic units capable of producing magnetic fields and an array of transportation electrodes capable of producing traveling-wave DEP forces are co-fabricated on a solid substrate (the second substrate). The second substrate supporting both electromagnetic unit array and transportation electrode array is bound to or attached to the first substrate to form a three-force chip.

<u>Modes of Carrying Out the Invention</u>

This invention is directed to an apparatus, also called a multiple-force chip (MFC), that comprises multiple types of built-in elements and is capable of producing different types of physical fields and thus different types of forces for manipulating, handling and processing particles and molecules on a chip, *e.g.*, biochip. On the one hand, the MFC may be more sophisticated and complicated in its structural arrangement and may require complex fabrication protocols in comparison with the currently available biochips such as electronic chip capable of generating DEP force, or electronic chip capable of generating electrophoresis force, or magnetic chip capable of generating magnetic forces. On the other hand, MFC provides a completely new way for processing biological samples and for performing chemical, biological or biochemical reactions.

(1) Typical currently available biochip can handle or process one type of particles and molecules. For example, electrophoresis-based chip can manipulate charged molecules or particles; an electromagnetic chip can handle only magnetic particles.

But a single MFC can process or handle particles or molecules of many different types, such as magnetic particles, electrically-charged or non-charged particles or molecules, dielectrically-polarizable particles or molecules. This provides an additional flexibility for the design and development of biochip based bioassay.

(2) Many restrictions and side-effects associated with current, single-type-of-force generating chips can be overcome by MFC. For example, typically, an electrophoresis chip can process only those liquid solutions whose electronic conductivity is very low. Thus, integrating multiple-step reactions on an electrophoresis chip is difficult, if not impossible, simply because a number of biochemical reaction buffers demand high-electric conductivity solutions. An MFC may be readily designed for such integration since the MFC can be operated not only with electrophoresis forces for those bioreactions in which electric conductivity of the solutions involved is low and but also with other types of forces, *e.g.*, magnetic or acoustic forces, for the bio-reactions in which electrical conductivity of the solutions involved is high.

(3) The MFC allows for sequential and/or simultaneous application of multiple types of physical forces produced on microparticles or molecules in a liquid suspension. The combination of these forces in 3-dimensional space can increase the MFC's discriminatory capabilities in selective manipulation, separation and isolation of target particles.

The moieties, *e.g.*, molecules and particles, that can be manipulated by the multiple-force chips of the present invention include many types of particles – solid (*e.g.*: glass beads, latex particles, magnetic beads), liquid (*e.g.*: liquid droplets) or gaseous particles (e.g.: gas bubble), dissolved particles (*e.g.*: molecules, proteins, antibodies, antigens, lipids, DNAs, RNAs, molecule-complexes), suspended particles (*e.g.*: glass beads, latex particles, polystyrene beads), particle-molecule complexes (*e.g.*: DNA molecule-magnetic bead complexes formed by immobilizing DNA molecules on magnetic bead surfaces, or protein molecule-polystyrene bead complexes formed by covering polystyrene bead surfaces with protein molecules). Particles can be organic (*e.g.*: mammalian cells or other cells, bacteria, virus, or other microorganisms) or inorganic (*e.g.*, metal particles). Particles can be of different shapes (*e.g.*, sphere, elliptical sphere, cubic, discoid, needle-type) and can be of different sizes (*e.g.*: nano-meter-size gold sphere, to micrometer-size cells, to millimeter-size particle-aggregate). Examples of particles include, but are not limited to, biomolecules

such as DNA, RNA, chromosomes, protein molecules (*e.g.*: antibodies), cells, colloid particles (*e.g.* polystyrene beads, magnetic beads), molecule-particle complexes (*e.g.*, protein molecules bound to antibody-coated magnetic beads).

It is particularly important to note that a single multiple-force chip may have the capabilities to manipulate different types of particles either simultaneously or sequentially. For example, an multiple-force chip may process biomolecule-coated magnetic beads and electrically-charged cells or molecules simultaneously if the chip has incorporated magnetic-force-generating elements and electrophoretic-force-generating elements.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections that follow.

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

As used herein, "a" or "an" means "at least one" or "one or more."

As used herein, "chip" refers to a solid substrate with a plurality of one-, two- or three-dimensional micro structures or micro-scale structures on which certain processes, such as physical, chemical, biological, biophysical or biochemical processes, etc., can be carried out. The micro structures or micro-scale structures such as, channels and wells, electrode elements, electromagnetic elements, are incorporated into, fabricated on or otherwise attached to the substrate for facilitating physical, biophysical, biological, biochemical, chemical reactions or processes on the chip. The chip may be thin in one dimension and may have various shapes in other dimensions, for example, a rectangle, a circle, an ellipse, or other irregular shapes. The size of the major surface of chips of the present invention can vary considerably, *e.g.*, from about 1 mm^2 to about 0.25 m^2. Preferably, the size of the chips is from about 4 mm^2 to about 25 cm^2 with a characteristic

dimension from about 1 mm to about 5 cm. The chip surfaces may be flat, or not flat. The chips with non-flat surfaces may include channels or wells fabricated on the surfaces.

As used herein, "physical field," *e.g.*, used itself or used as "physical field in a region of space" or "physical field is generated in a region of space" means that the region of space has following characteristics. When a moiety of appropriate properties is introduced into the region of space (i.e. into the physical field), forces are produced on the moiety as a result of the interaction between the moiety and the field. A moiety can be manipulated within a field via the physical forces exerted on the moiety by the field. Exemplary fields include electric, magnetic, acoustic, optical and velocity fields. In the present invention, physical field always exists in a medium in a region of space, and the moiety to be manipulated is suspended in, or is dissolved in, or more generally, is placed in the medium. Typically, the medium is a fluid such as aqueous or non-aqueous liquids, or a gas. Depending on the field configuration, an electric field may produce electrophoretic forces on charged moieties, or may produce conventional dielectrophoretic forces and/or traveling wave dielectrophoretic forces on charged and/or neutral moieties. Magnetic field may produce magnetic forces on magnetic moieties. Acoustic field may produce acoustic radiation forces on moieties. Optical field may produce optical radiation forces on moieties. Velocity field in the medium in a region of space refers to a velocity distribution of the medium that moves in the region of the space. Various mechanisms may be responsible for causing the medium to move and the medium at different positions may exhibit different velocities, thus generating a velocity field. Velocity field may exert mechanical forces on moieties in the medium.

As used herein, "medium (or media)" refers to a fluidic carrier, e.g., liquid or gas, wherein a moiety is dissolved, suspended or contained.

As used herein, "microfluidic application" refers to the use of microscale devices, *e.g.*, the characteristic dimension of basic structural elements is in the range between less than 1 micron to 1 cm scale, for fluidic manipulation and process, typically for performing specific biological, biochemical or chemical reactions and procedures. The specific areas include, but are not limited to, biochips, *i.e.*, chips for biologically related reactions and processes, chemchips, *i.e.*, chips for chemical reactions, or a combination thereof.

As used herein, "built-in structures on said substrate" means that the structures are built into the said substrate or the structures are located on the substrate and the structures are structurally linked to the substrate. In one embodiment, the built-in structures may be

fabricated on the said substrate. For example, as described in "Dielectrophoretic manipulation of cells using spiral electrodes by Wang et al., *Biophys. J.*, 72:1887-1899 (1997)", spiral electrodes are fabricated on a glass substrate. Here the spiral electrodes are "built-in" structures on the glass substrate. In another embodiment, the "built-in" structures may be first fabricated on one substrate and the structure-containing first substrate may then be attached or bound to a second substrate. Such structures are "built-in" structures not only on the first substrate but also on the second substrate. In still another embodiment, the built-in structures may be attached or bound to the said substrate. For example, thin, electrically-conductive wires may be used as electrodes for producing electric field. These electric wires may be bound or attached to a glass substrate. In this case, the electrically-conductive wires are "built-in" structures on the glass substrate. Throughout this application, when it is described that "built-in" structures on the chip or on the substrate are capable of generating physical forces and/or physical fields or these structures generate physical forces and/or physical fields, these structures are used in combination with external signal sources or external energy sources.

As used herein, "structures internal to said apparatus" means that the structures are integral parts of and structurally linked to other parts of the apparatus, or the structures are not separated or separable from other structural elements of the apparatus. For example, such internal structures can be microfabricated or otherwise attached to the substrate or other structural element(s) of the apparatus. Any "built-in structures on said substrates" described above are "structures internal to said apparatus" as long as the said apparatus comprise the substrates. Any built-in structures on a chip are "structures internal to said apparatus" as long as the said apparatus comprise the chip. Throughout this application, when it is described that "internal" structures of apparatus are capable of generating physical forces and/or physical fields or these structures generate physical forces and/or physical fields, these structures are used in combination with external signal sources or external energy sources.

As used herein, "micro-scale structures" means that the scale of the internal structures of the apparatus for exerting desired physical forces must be compatible with and useable in microfluidic applications and have characteristic dimension of basic structural elements in the range from about 1 micron to about 20 mm scale.

As used herein, "moiety" refers to any substance whose manipulation in a chip format is desirable. Normally, the dimension of the moiety should not exceed 1 cm. For example,

if the moiety is spherical or approximately spherical, the dimension of the moiety refers to the diameter of the sphere or an approximated sphere for the moiety. If the moiety is cubical or approximately cubical, then the dimension of the sphere refers to the side width of the cube or an approximated cube for the moiety. If the moiety has an irregular shape, the dimension of the moiety may refer to the average between its largest axis and smallest axis. Non-limiting examples of moieties that can be manipulated through the present methods include cells, cellular organelles, viruses, particles, molecules, *e.g.*, proteins, DNAs and RNAs, or an aggregate or complex thereof.

Moiety to be manipulated includes many types of particles – solid (*e.g.*: glass beads, latex particles, magnetic beads), liquid (*e.g.*: liquid droplets) or gaseous particles (e.g.: gas bubble), dissolved particles (*e.g.*: molecules, proteins, antibodies, antigens, lipids, DNAs, RNAs, molecule-complexes), suspended particles (*e.g.*: glass beads, latex particles, polystyrene beads). Particles can be organic (*e.g.*: mammalian cells or other cells, bacteria, virus, or other microorganisms) or inorganic (*e.g.*, metal particles). Particles can be of different shapes (*e.g.*, sphere, elliptical sphere, cubic, discoid, needle-type) and can be of different sizes (*e.g.*: nano-meter-size gold sphere, to micrometer-size cells, to millimeter-size particle-aggregate). Examples of particles include, but not limited to, biomolecules such as DNA, RNA, chromosomes, protein molecules (*e.g.*: antibodies), cells, colloid particles (*e.g.* polystyrene beads, magnetic beads), any biomolecules (e.g. enzyme, antigen, hormone etc). One specific type of particle refers to complexes formed between moieties and their binding partners, as described in a co-pending US patent application entitled "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US patent application no. 09/636,104, by Wang et al., filed on August 10, 2000). The examples of such complexes include particle-particle complexes, particle-molecule complexes (e.g.: cell-magnetic bead complexes formed by binding of the cells onto antibody-coated beads through the interaction between the antigens or protein molecules on cell surfaces and the antibody molecules immobilized on the magnetic bead surfaces; DNA molecule-magnetic bead complexes formed by immobilizing DNA molecules on magnetic bead surfaces, or protein molecule-polystyrene bead complexes formed by covering polystyrene bead surfaces with protein molecules). The methods disclosed in a co-pending US patent application "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US patent application no. 09/636,104, by Wang et al., filed on August 10, 2000) may be used for manipulating moieties and/or binding partner-moiety

complexes in the devices and apparatus in the present invention. The co-pending US patent application "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US patent application no. 09/636,104) by Wang et al, filed on August 10, 2000 is incorporated by reference in their entirety.

As used herein, "plant" refers to any of various photosynthetic, eucaryotic multi-cellular organisms of the kingdom Plantae, characteristically producing embryos, containing chloroplasts, having cellulose cell walls and lacking locomotion.

As used herein, "animal" refers to a multi-cellular organism of the kingdom of Animalia, characterized by a capacity for locomotion, nonphotosynthetic metabolism, pronounced response to stimuli, restricted growth and fixed bodily structure. Non-limiting examples of animals include birds such as chickens, vertebrates such fish and mammals such as mice, rats, rabbits, cats, dogs, pigs, cows, ox, sheep, goats, horses, monkeys and other non-human primates.

As used herein, "bacteria" refers to small prokaryotic organisms (linear dimensions of around 1 micron) with non-compartmentalized circular DNA and ribosomes of about 70S. Bacteria protein synthesis differs from that of eukaryotes. Many anti-bacterial antibiotics interfere with bacteria proteins synthesis but do not affect the infected host.

As used herein, "eubacteria" refers to a major subdivision of the bacteria except the archaebacteria. Most Gram-positive bacteria, cyanobacteria, mycoplasmas, enterobacteria, pseudomonas and chloroplasts are eubacteria. The cytoplasmic membrane of eubacteria contains ester-linked lipids; there is peptidoglycan in the cell wall (if present); and no introns have been discovered in eubacteria.

As used herein, "archaebacteria" refers to a major subdivision of the bacteria except the eubacteria. There are three main orders of archaebacteria: extreme halophiles, methanogens and sulphur-dependent extreme thermophiles. Archaebacteria differs from eubacteria in ribosomal structure, the possession (in some case) of introns, and other features including membrane composition.

As used herein, "virus" refers to an obligate intracellular parasite of living but non-cellular nature, consisting of DNA or RNA and a protein coat. Viruses range in diameter from about 20 to about 300 nm. Class I viruses (Baltimore classification) have a double-stranded DNA as their genome; Class II viruses have a single-stranded DNA as their genome; Class III viruses have a double-stranded RNA as their genome; Class IV viruses have a positive single-stranded RNA as their genome, the genome itself acting as mRNA;

Class V viruses have a negative single-stranded RNA as their genome used as a template for mRNA synthesis; and Class VI viruses have a positive single-stranded RNA genome but with a DNA intermediate not only in replication but also in mRNA synthesis. The majority of viruses are recognized by the diseases they cause in plants, animals and prokaryotes. Viruses of prokaryotes are known as bacteriophages.

As used herein, "fungus" refers to a division of eucaryotic organisms that grow in irregular masses, without roots, stems, or leaves, and are devoid of chlorophyll or other pigments capable of photosynthesis. Each organism (thallus) is unicellular to filamentous, and possesses branched somatic structures (hyphae) surrounded by cell walls containing glucan or chitin or both, and containing true nuclei.

As used herein, "binding partners" refers to any substances that both bind to the moieties with desired affinity or specificity and are manipulatable with the desired physical force(s). Non-limiting examples of the binding partners include cells, cellular organelles, viruses, particles, microparticles or an aggregate or complex thereof, or an aggregate or complex of molecules.

As used herein, "microparticles" refers to particles of any shape, any composition, any complex structures that are manipulatable by desired physical force(s) in microfluidic settings or applications. One example of microparticles is magnetic beads that are manipulatable by magnetic forces. Another example of microparticles is a cell that are manipulatable by an electric force such as a traveling-wave dielectrophoretic force. The microparticles used in the methods could have a dimension from about 0.01 micron to about ten centimeters. Preferably, the microparticles used in the methods have a dimension from about 0.01 micron to about several thousand microns. Examples of the microparticles include, but are not limited to, plastic particles, polystyrene microbeads, glass beads, magnetic beads, hollow glass spheres, particles of complex compositions, microfabricated free-standing microstructures, *etc.* Other particles include cells, cell organelles, large biomolecules such as DNA, RNA and proteins etc.

As used herein, "manipulation" refers to moving or processing of the moieties, which results in one-, two- or three-dimensional movement of the moiety, in a chip format, whether within a single chip or between or among multiple chips, or on a substrate or among substrates of an apparatus. "Manipulation" of moieties can also be performed in liquid containers. Non-limiting examples of the manipulations include transportation, focusing, enrichment, concentration, aggregation, trapping, repulsion, levitation,

separation, fractionation, isolation or linear or other directed motion of the moieties. For effective manipulation, the characteristics of the moiety to be manipulated and the physical force used for manipulation must be compatible. For example, moiety with certain magnetic properties can be used with magnetic force. Similarly, moiety with electric charge(s) can be used with electrostatic (i.e. electrophoretic) force. In the case of manipulating binding partner-moiety complexes, the characteristics of the moiety, or its binding partner, and the physical force used for manipulation must be compatible. For example, moiety or its binding partner with certain dielectric properties to induce dielectric polarization in the moiety or its binding partner can be used with dielectrophoresis force.

As used herein, "the moiety is not directly manipulatable" by a particular physical force means that no observable movement of the moiety can be detected when the moiety itself not coupled to a binding partner is acted upon by the particular physical force.

As used herein, "physical force" refers to any force that moves the moieties or their binding partners without chemically or biologically reacting with the moieties and the binding partners, or with minimal chemical or biological reactions with the binding partners and the moieties so that the biological/chemical functions/properties of the binding partners and the moieties are not substantially altered as a result of such reactions. Throughout the application, the term of "forces" or "physical forces" always means the "forces" or "physical forces" exerted on a moiety or moieties. The "forces" or "physical forces" are always generated through "fields" or "physical fields". The forces exerted on moieties by the fields depend on the properties of the moieties. Thus, for a given field or physical field to exert physical forces on a moiety, it is necessary for the moiety to have certain properties. While certain types of fields may be able to exert forces on different types of moieties having different properties, other types of fields may be able to exert forces on only limited type of moieties. For example, magnetic field can exert forces or magnetic forces only on magnetic particles or moieties having certain magnetic properties, but not on other particles, *e.g.*, polystyrene beads. On the other hand, a non-uniform electric field can exert physical forces on many types of moieties such as polystyrene beads, cells, and also magnetic particles. It is not necessary for the physical field to be able to exert forces on different types of moieties or different moieties. But it is necessary for the physical field to be able to exert forces on at least one type of moiety or at least one moiety.

As used here in, "electric forces (or electrical forces)" are the forces exerted on moieties by an electric (or electrical) field.

As used herein, "magnetic forces" are the forces exerted on moieties by a magnetic field.

As used herein, "acoustic forces (or acoustic radiation forces)" are the forces exerted on moieties by an acoustic field.

As used herein, "optical (or optical radiation) forces" are the forces exerted on moieties by an optical field.

As used herein, "mechanical forces" are the forces exerted on moieties by a velocity field.

As used herein, "the moiety to be manipulated is substantially coupled onto surface of the binding partner" means that a certain percentage, and preferably a majority, of the moiety to be manipulated is coupled onto surface of the binding partner and can be manipulated by a suitable physical force via manipulation of the binding partner. Ordinarily, at least 0.5% of the moiety to be manipulated is coupled onto surface of the binding partner. Preferably, at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the moiety to be manipulated is coupled onto surface of the binding partner. The percentage of the coupled moiety includes the percentage of the moiety coupled onto surface of a particular type of binding partner or a plurality of binding partners. When a plurality of binding partners is used, the moiety can be coupled onto surface of the plurality of binding partners simultaneously or sequentially.

As used herein, "the moiety to be manipulated is completely coupled onto surface of the binding partner" means that at least 90% of the moiety to be manipulated is coupled onto surface of the binding partner. Preferably, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the moiety to be manipulated is coupled onto surface of the binding partner. The percentage of the coupled moiety includes the percentage of the moiety coupled onto surface of a particular type of binding partner or a plurality of binding partners. When a plurality of binding partners is used, the moiety can be coupled onto surface of the plurality of binding partners simultaneously or sequentially.

As used herein, "intracellular moiety" refers to any moiety that resides or is otherwise located within a cell, *i.e.*, located in the cytoplasm or matrix of cellular organelle, attached to any intracellular membrane, resides or is otherwise located within periplasma, if

there is one, or resides or is otherwise located on cell surface, *i.e.*, attached on the outer surface of cytoplasm membrane or cell wall, if there is one.

B. Chips and Apparatuses

In one aspect, the invention provides a chip for generating fields, which chip comprises: a) a substrate; and b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field. Here the physical fields are generated through a combination of the external energy source ("external" is with respect to the chip of the present invention) and the built-in structure located on the substrate or the chip. The external energy sources are connected to the built-in structures for energizing the built-in structures to generate a physical field such as electric field, magnetic field, acoustic field, optical field, or velocity field. Alternatively, the external energy source may provide signals of appropriate form (e.g., electrical signal, or optical signal or optical radiation). These signals from the external sources are then coupled to or linked to the built-in structures located on the substrate or the chip for producing physical fields such as electric field, magnetic field, acoustic field, optical field or velocity field.

The chip of the present invention can comprise a single substrate. Alternatively, the chip can comprise a plurality of structurally linked substrates. For example, an electrode array may be fabricated on one substrate and a microelectromagnetic unit may be fabricated on a second substrate. The electrode array, in combination with electrical signal sources, is capable of producing DC or AC electric fields. The electromagnetic unit array, in combination with electrical signal sources, is capable of producing magnetic fields. The two substrates may be bound together to form one chip with the electrode substrate and the electromagnetic substrate at the top and bottom respectively. These two substrates are structurally linked substrates. Such a step may be repeated to form chips comprising more than two structurally linked substrates.

In another example, two substrates may be linked together in series and bound to a third substrate. These two substrates are structurally linked substrates. Any suitable solid substrate can be used in the present chips. For example, the substrate material can be silicon (with a silicon dioxide or silicon nitride surface or other thin dielectric layer surfaces), plastic, glass, ceramics, rubber or polymer. The substrate material can be porous or non-porous.

Although the at least two different types of structures of the chip of the present invention can generate single or multiple types of fields, the chip is preferably designed so that the built-in structures of the chip generate at least two different types of physical fields. It is not necessary that the number of the built-in structures correspond to the number of the types of the physical fields generated via the built-in structures. However, it is preferable that the number of the built-in structures correspond to the number of the types of the physical fields so generated. For example, the chip can comprise two different types of built-in structures that are capable of generating two different types of physical fields, or three different types of built-in structures that are capable of generating three different types of physical fields, or four different types of built-in structures that are capable of generating four different types of physical fields, or more than four different types of built-in structures that are capable of generating more than four different types of physical fields.

The built-in structures can take any suitable forms in the chip. For example, the built-in structures can be in the form of single units, which can be located in a portion of or in entire chip. Alternatively, the built-in structures can comprise a plurality of microunits. Such microunits can be, partially or completely, individually addressable or interconnected. When a plurality of microunits are used in the chip, the chip can preferably further comprise means for selectively energizing any one of the plurality of microunits.

The chips of the present invention can be designed to generate any type of desired physical field(s), and preferably at least two different types of physical fields. In one specific embodiment, the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic, optical and velocity fields. In another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic, and optical fields. In still another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, optical fields, and velocity fields. In still another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, acoustic and optical fields, and velocity fields. In still another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic fields, and velocity fields. In still another specific embodiment, the built-in structures are capable of

generating at least two different types of physical fields selected from the group consisting of magnetic, acoustic and optical fields, and velocity fields. In yet another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of optical field and a non-uniform AC electric field. In yet another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of standing wave acoustic field and a uniform electrostatic field. In yet another specific embodiment, the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of electric field and velocity field.

Electric fields may be generated by using built-in electrode structures on a substrate or on a chip, which are energized by electrical signal sources. The built-in structures are the electrode elements and electrode arrays that are incorporated on a chip. When the appropriately designed electrode elements and electrode arrays are energized by electrical signal sources, electric (or electrical) fields are generated in the regions around the chip. Electric field may be divided into DC electric field and AC electric field according to whether the field remains in one direction or whether the field direction alternates with time. Electric field may also be divided into uniform field or non-uniform field. Here "uniform field" or "non-uniform field" is in terms of the field distributed in space. For a non-uniform AC electric field of single harmonics (e.g. the field change with time follows a sinusoidal dependency with frequency varying from practically DC such as 0.00001 Hz to several hundred GHz such as 2 GHz), the field magnitude may have a non-uniform distribution, and/or the field phase may have a non-uniform distribution. The AC field may have multiple frequency components. The field may be modulated in magnitude, or modulated in frequency. The field change with time may follow other dependency function other than sinusoidal. Examples of the electrode structures or electrode elements for producing electric fields include, but not limited to, the following, planner electrodes covering the whole surface of the substrate or a large surface area of the substrate ("large" means, in a single dimension, at least ten times larger than the size of moiety to be manipulated if the field is generated for manipulation), interdigitated castellated electrodes, parallel electrodes, spiral electrodes, comb type electrodes, polynomial electrodes, individually addressable electrode array with electrode elements being circle, square, diamond or many other regular or irregular shapes. The following articles provide many examples of the electrode structures that may be used to generate electric fields: Gale *et al.*,

IEEE Trans. Biomedical Engineering 45: 1459-1469 (1998); Wang, *et al., Biochim. Biophys. Acta.* 1243:185-194 (1995); Wang, *et al., IEEE Trans. Ind. Appl.* 33:660-669 (1997); Wang, *et al., Biophys. J.* 72:1887-1899 (1997); Wang, *et al., Biophys. J.* 74:2689-2701 (1998), Huang, *et al., Biophys. J.* 73:1118-1129 (1997) and Yang, *et al., Anal. Chem.* 71(5):911-918 (1999); Gascoyne, *et al., IEEE Trans. Ind. Apps.* 33(3):670-678 (1997), Becker, *et al., Proc. Natl. Acad. Sci. USA* 92:860-864 (1995) and Becker, *et al., J. Phys. D: Appl. Phys.* 27:2659-2662 (1994); Huang, *et al., J. Phys. D: Appl. Phys.* 26:1528-1535 (1993) ; Wang, *et al., J. Phys. D: Appl. Phys.* 26:1278-1285 (1993);Hawkes, *et al., Microbios.* 73:81-86 (1993); and Cheng, *et al., Nat. Biotech.* 16:547-546 (1998)); Stephens, *et al., Bone Marrow Transplantation* 18:777-782 (1996)); Washizu, *et al., IEEE Trans. Ind. Appl.* 30:835-843 (1994); Green and Morgan, *J. Phys. D: Appl. Phys.* 30:L41-L44 (1997); Hughes, *et al., Biochim. Biophys. Acta.* 1425:119-126 (1998); and Morgan, *et al., Biophys. J.* 77:516-525 (1999)); Fuhr, *et al., Biochim. Biophys. Acta.* 1108:215-233 (1992)); Washizu, *et al., IEEE Trans. Ind. Appl.* 26:352-358 (1990); Fiedler, *et al., Anal. Chem.* 70:1909-1915 (1998); and Müller, *et al., Biosensors and Bioelectronics* 14:247-256 (1999)); Schnelle, *et al., Biochim. Biophys. Acta.* 1157:127-140 (1993); Fiedler, *et al.* (1995); Fuhr, *et al.* (1995a); Fiedler, *et al.* (1998); Müller, *et al.* (1999)); Hagedorn, *et al., Electrophoresis* 13:49-54 (1992); Fuhr, *et al., Sensors and Actuators A:* 41:230-239 (1994); Morgan, *et al., J. Micromech. Microeng.* 7:65-70 (1997); . Schnelle T., *et al.*, in *Biochim. Biophys. Acta.* 1157:127-140, 1993, pages; Müller, T., *et al.*, in *Biosensors and Bioelectronics,* 14:247-256, (1999); Fuhr, G., *et al.*, in *Cellular Engineering.* Autumn: 47-57, (1995); Fiedler S. *et al.*, in . *Microsystem Technologies.* 2: 1-7, 1995; Fiedler, S., *et al., Anal. Chem.* 70: 1909-1915, (1998).

Electrode elements, electrode structures, electrode arrays may be fabricated into the substrates using many different fabrication methods, as known to those skilled in the art of microlithography and microfabrication (*See, for example,* Rai-Choudhury P. (Editor), Handbook of Microlithography, Micromachining and Microfabrication, Volume 2: Micromachining and microfabrication. SPIE Optical Engineering Press, Bellingham, Washington, USA (1997)). In many cases, standard microfabrication and micromachining methods and protocols may be used. One example of the fabrication methods is photolithography involving single or multiple photomasks. The protocols in the microfabrication may include many basic steps, for example, photolithographic mask generation, metal deposition, insulator deposition, photoresist deposition, photoresist

patterning with masks and developers, metal or insulator layer patterning. Electrodes may be made of metal materials such as aluminum, gold, silver, tin, copper, platinum, palladium and carbon, semiconductor materials such as phosphorous-doped silicon, and any other materials as along as they conduct electric currents. The substrate on which the electrodes are fabricated may be silicon, plastic, glass, ceramics or other solid materials. The solid materials may be porous or non-porous. Those who are skilled in microfabrication and micromachining fabrication may readily choose and determine the fabrication protocols and materials that are used for fabrication of particular electrode structures.

Magnetic field may be generated with various approaches. For example, the electromagnetic chip disclosed in the co-pending U.S. Patent Application Serial No. 09/399, 299, filed September 16, 1999, which is incorporated by reference in its entirety, can be used. Typically, such electromagnetic chips with individually addressable micro-electromagnetic units comprise: a substrate; a plurality of micro-electromagnetic units on the substrate, each unit capable of inducing magnetic field upon applying electric current; means for selectively energizing any one of a plurality of units to induce a magnetic field therein. Preferably, the electromagnetic chips further comprise a functional layer coated on the surface of the chips for immobilizing certain types of moieties or molecules. In this example, microelectromagnetic units are the built-in structures located on the chip or the substrate and the electrical current source that is connected to the microelectromagnetic units is the external energy sources. When the electric current from the external current source is applied to the microelectromagnetic units, magnetic fields will be generated in the regions around the microelectromagnetic units. The electromagnetic units of other geometries and structures may also be used. The examples of such electromagnetic units include, but are not limited to, the following: Ahn, C., *et al.*, *J. Microelectromechanical Systems*. Volume 5: 151-158 (1996); Ahn, C., *et al.*, *IEEE Trans. Magnetics*. Volume 30: 73-79 (1994); Liakopoulos *et al.*, *in Transducers 97,* pages 485-488, presented in 1997 International Conference on Silid-State Sensors and Actuators, Chicago, June 16-19, 1997; U.S. Patent No. 5,883,760 by Naoshi *et al.*. The above publications, and the co-pending U.S. Patent Application Serial No. 09/399, 299, filed September 16, 1999, further disclose the materials, methods and protocols that may be used to fabricate the electromagnetic structures on a chip.

Acoustic fields may be generated with various approaches. For example, piezoelectric transducers may be incorporated into, or fabricated into a substrate, and may

be used for producing acoustic fields. One example is to use piezoelectric ceramic discs as substrates and both surfaces of the ceramic discs are covered with metal thin film electrodes. Another example is to use piezoelectric ceramic discs as substrates and the surfaces of the ceramics are deposited and processed with electrode arrays of different geometries. Another example is to use a non-piezoelectric substrate and one surface of the substrates is etched with an array of wells of appropriate sizes. The electrode materials and piezoelectric materials are deposited into the etched wells to form piezoelectric transducers with sandwich structures of electrode/piezoelectric material/electrode. Another example is to use the phased array of piezoelectric transducers described in U.S. Patent 6,029,518 by Oeftering, R.. In all these examples, the piezoelectric transducers including piezoelectric material plus the electrodes are the built-in structures on the substrate or on the chip. When electrical signals from an external signal source are connected to the electrodes, the piezoelectric transducers are energized to produce mechanical displacements that can be coupled into the medium that surrounds the substrate and produce acoustic-wave fields in the medium. Depending on the configuration of the substrate and the structures that are built around the substrates, different types of acoustic fields may be induced. A standing wave acoustic field may be produced in a chamber comprising a piezoelectric substrate and an acoustic wave reflector plate that are separated by an appropriate distance (e.g. Yasuda K. et al, *J. Acoust. Soc. Am.* Vol. 102 (1), p642-645, July, 1997; Yasuda K. and Kamakura T. *Appl. Phys. Lett,* Vol. 71(13), p1771-1773, Sep. 1997). In another example, a traveling wave acoustic field may be used. In still another example, the acoustic field may have both standing wave as well as traveling wave field components.

There are various approaches for producing optical field. In one example, the built-in structures are the optical elements and arrays that are incorporated on a substrate or a chip and the external energy source is an optical signal source (*e.g.*, a laser source). When the light produced by the optical signal sources passes through the built-in optical elements and arrays in the chip or substrate, optical fields are generated in the regions around the chip and the optical field distribution is dependent on the geometrical structures, sizes and compositions of the built-in optical elements and arrays. In another example, the built-in structures are the electro-optical elements and arrays that are incorporated on a chip and the external energy sources are electrical signal sources (*e.g.*, a DC current source, or an AC current source). When the electrical signals from the external electrical signal sources are

applied to the built-in electro-optical elements and arrays, light is produced from these elements and arrays and optical fields are generated in the regions around the chip.

Velocity field in the medium in a region of space refers to a velocity distribution of the medium that moves in the region of the space. Various approaches may be used to induce the medium to move, resulting in a velocity field. In one case, the substrate surfaces are processed to have appropriate surface charges or appropriate surface charge distribution. The substrate may be used together with other structures (e.g. a cover plate, a gasket or spacer) to form an enclosed chamber with various input/output ports. After a liquid medium is introduced into the chamber, external electrical signal sources may be used to apply a voltage across certain directions in the chamber. The voltage-resultant electric field may interact with the surface charges on the substrate (and/or the surface charges on other surfaces of the chamber) to induce a fluid flow in the liquid medium. The resultant velocity distribution in the positions within the chamber is a velocity field.

In another example, the velocity field may be produced by generating a thermal gradient or temperature gradient in the medium. A single, or multiple heating or cooling elements may be fabricated into a substrate. The heating elements may be thin film resistors or thin wire resistors (e.g. metal film or metal wire). The cooling elements may be a Peltier effect junction or a Peltier element. These heating and cooling elements can be energized by external electrical signal sources. Electrical current from external signal sources may be applied to a metal film, or a metal wire to produce "heat", serving as a heating element. Electrical current from external signal sources may be applied to a Peltier element to increase or decrease the temperature. Such heating and/or cooling process will result in a temperature gradient in the medium that is in direct contact or in indirect contact with the heating and/or cooling elements. The temperature gradient will result in motion of the medium, leading to a velocity field.

In another example, velocity field may be produced by using the electro-mechanical elements/devices that are incorporated on a chip. The external energy sources may be electrical signal sources (*e.g.*, a DC current source). The electromechanical device may be a microfabricated pump (e.g. electromagnetic) that can generate pressures to pump fluids. The electromechanical device may be a microfabricated valve that can open and close, resulting in the motion of fluids. The electromechanical elements/devices may be an array of microfabricated capillaries or tips. These capillaries or tips are covered with functional materials such as temperature sensitive materials, surface memory alloy. These capillaries

or tips can be further connected with external signal sources or coupled with external physical forces, which will cause the motion of the capillaries or tips when energized appropriately. When appropriately designed and fabricated electro-mechanical elements/devices on the chip are energized by electrical signal sources, these elements/devices, or component(s) within these elements/devices exhibit certain motion, causing the medium that is in direct contact or in indirect contact with the electro-mechanical elements to move. The resultant velocity distribution within the medium is a velocity field.

Other types of physical fields may be generated by the built-in structures in the substrate or in the chip. All these physical fields have the characteristics that when moieties, or particles, or binding partner-moiety complexes that have appropriate properties are placed into the physical fields, forces are exerted on these moieties, particles, or binding partner-moiety complexes.

The scale of the built-in structures for generating desired physical fields must be compatible with and useable in microfluidic applications. For example, the built-in structures can be micro-scale structures. Normally, the micro-scale structures have characteristic dimension of basic structural elements in the range from about 0.1 micron to about 20 mm scale. Preferably, the micro-scale structures have characteristic dimension of basic structural elements in the range from about 1 micron to about 1 mm scale.

The present chip can be used in a wide variety of applications in chemical/ biochemical/ biological reactions, processes and procedures. These reactions, processes and procedures may be used for biomedical research, clinical diagnosis and pharmaceutical drug development. The chip in the present invention may be used for many processes, such as manipulating moieties, facilitating chemical/ biochemical/ biological reactions, controlling chemical/ biochemical/ biological processes (e.g. temperature control within a reaction chamber), and controlling fluidic flow. The built-in structures or built-in elements on the chip may be designed to suit its particular uses. A variety of chips having different built-in structures may be fabricated or made for different applications. In addition to the built-in structures and/or elements described above for producing physical fields, the chip may contain other structural elements that suit its particular uses, or the chip may be used with other elements. For example, if the chip is used for moiety manipulation, the chip may be used with structural elements such as cover plates, spacers, gaskets, and input and output ports so that the constructed apparatus is suitable for supporting or holding a moiety

to be manipulated. The chip may further comprise a functional layer on the support for immobilizing a moiety on the support.

In another aspect, the present invention is directed to a combination, which combination comprises: a) at least two chips each of which comprises a substrate and at least two different types of structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field; and b) means for transporting a moiety to be manipulated between said chips. In such cases, each chip can comprise at least two different structures described above, and each of these structures when used in combination with an external energy source, can generate one type of physical field such as electric field, magnetic field, acoustic field, optical field and velocity field, and other fields.

In still another aspect, the present invention is directed to an apparatus for manipulating a moiety, which apparatus comprises a substrate for holding or supporting a moiety to be manipulated and at least two different types of structures internal to said apparatus, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field and thus producing one type of physical force on said moiety. The internal structures that are capable of generating physical field(s) and physical force(s) can be located on the substrate. Alternatively, the internal structures can be located off the substrate but still remain structurally an integral part of the apparatus.

Because the physical field in the present invention has the characteristic such that moieties of appropriate properties will experience physical forces when they are introduced into the physical fields, any chip of the present invention, described above, is an example of the apparatus of the present invention. Thus, the apparatus may comprise any of the chip for generating fields, which chip comprises: a) a substrate; and b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field.

The apparatus may comprise one substrate as long as the internal structures in the apparatus are capable of producing at least two types of physical forces on moiety (or moieties) introduced in the apparatus. The apparatus may comprise multiple structurally linked substrates. For example, an electrode array may be fabricated on one substrate and a microelectromagnetic unit may be fabricated on a second substrate. The electrode array, in

combination with electrical signal sources, is capable of producing DC or AC electric field and thus exerting electric forces (e.g. electrostatic force, and/or conventional dielectrophoretic force, and/or traveling-wave dielectrophoretic force) on neutral or charged moieties. The electromagnetic unit array, in combination with electrical signal sources, is capable of producing magnetic field and thus exerting magnetic forces on moieties that have certain magnetic properties. The two substrates may be bound together to form one combined substrate with the electrode substrate and the electromagnetic substrate at the top and bottom respectively. These two substrates are structurally linked substrates. Such a step may be repeated to form combined substrates comprising more than two structurally linked substrates.

In another example, two substrates may be linked together in series and bound to a third substrate. These two substrates are structurally linked substrates. Again, such a step may be repeated to form combined substrates comprising more than two structurally linked substrates.

In still another example, an electrode array may be fabricated on one substrate and a microelectromagnetic unit array may be fabricated on another substrate. The electrode array, in combination with electrical signal sources, is capable of producing DC or AC electric field and thus exerting electric forces (e.g. electrostatic force, and/or conventional dielectrophoretic force, and/or traveling-wave dielectrophoretic force) on neutral or charged moieties. The electromagnetic unit array, in combination with electrical signal sources, is capable of producing magnetic field and thus exerting magnetic forces on moieties that have certain magnetic properties. These two substrates may be used together to form an apparatus, i.e., a fluidic chamber in which the first, electrode substrate is used as the bottom plate of the chamber and the second, electromagnetic substrate is used as the top plate, and a gasket or a spacer is used to separate the two substrates. These two substrates are structurally linked substrates in an apparatus. The apparatus (i.e. the fluidic chamber) may further comprise input ports and output ports to allow the introduction of (or removal of) the moiety into (out of) the fluidic chamber.

In still another example, an electrode array may be fabricated on a substrate. The electrode array, in combination with electrical signal sources, is capable of producing DC or AC electric field and thus exerting electric forces (e.g. electrostatic force, and/or conventional dielectrophoretic force, and/or traveling-wave dielectrophoretic force) on neutral or charged moieties. A microfabricated array of lenses or optical filters or other

optical elements may be fabricated on another substrate. The fabricated array of optical elements, in combination with external optical signal source, is capable of producing an optical radiation field and thus exerting optical radiation forces on moieties. These two substrates may be used together to form an apparatus, i.e., a fluidic chamber in which the first substrate comprising electrode array is used as the bottom plate of the chamber and the second substrate comprising optical array is used as the top plate, and a gasket or a spacer is used to separate the two substrates. These two substrates are structurally linked substrates. The apparatus (i.e. the fluidic chamber) may further comprise input ports and output ports to allow the introduction of (or removal of) the moiety into (out of) the fluidic chamber.

Any suitable solid substrate can be used in the present apparatuses. For example, the substrate material can be silicon (with a silicon dioxide or silicon nitride surface or other thin dielectric layer surfaces), plastic, glass, ceramics, rubber or polymer. The substrate material can be porous or non-porous.

Although the at least two different types of internal structures of the apparatus of the present invention can generate single or multiple types of forces on moieties, the apparatus is preferably designed so that the internal structures of the apparatus generate at least two different types of physical forces. It is not necessary that the number of the internal structures correspond to the number of the types of the physical forces generated via the internal structures. However, it is preferable that the number of the internal structures corresponds to the number of the types of the physical forces so generated. For example, the apparatus can comprise two different types of internal structures that are capable of generating two different types of physical forces, or three different types of internal structures that are capable of generating three different types of physical forces, or four different types of internal structures that are capable of generating four different types of physical forces, or more than four different types of internal structures that are capable of generating more than four different types of physical forces. Multiple types of physical forces may exert on one moiety or one type of moiety. Alternatively, multiple types of physical forces may exert on multiple types of moieties. Thus, when an apparatus comprise two (three, four, or more than four) different types of internal structures that are capable of generating two (three, four, or more than four) different types of physical forces, it is not necessary for the two (three, four, or more than four) different types of physical forces exerted on same moiety or the same types of moieties.

It is important to note several points throughout the entirety of this application. When it is described that "internal" structures of apparatus or "built-in" structures on the chip are capable of generating physical forces and/or physical fields, or these structures generate physical forces and/or physical fields, these structures are used in combination with external signal sources or external energy sources. The term of "forces" or "physical forces" always means "forces" or "physical forces" exerted on a moiety or moieties. The "forces" or "physical forces" are always generated through "fields" or "physical fields". The forces exerted on moieties by the fields depend on the properties of the moieties. Thus, for a given field or physical field to exert physical forces on a moiety, it is necessary for the moiety have certain properties. While certain types of fields may be able to exert forces on different types of moieties having different properties, other types of fields may be able to exert forces on only limited type of moieties. For example, magnetic field can exert forces or magnetic forces only on magnetic particles or moieties having certain magnetic properties, but not on other particles *e.g.*, polystyrene beads. On the other hand, a non-uniform electric field can exert physical forces on many types of moieties such as polystyrene beads, cells, and also magnetic particles. It is not necessary for the physical field to be able to exert forces on different types of moieties or different moieties. But it is necessary for the physical field to be able to exert forces on at least one type of moiety or at least one moiety.

When the internal structures of the apparatus are located on the substrate, the internal structures can take any suitable forms on the substrate or built into the substrate. For example, the internal structures can be in the form of single units, which can be located in a portion of or in entire substrate. Alternatively, the internal structures can comprise a plurality of microunits. Such microunits can be, partially or completely, individually addressable or interconnected. When a plurality of microunits are used in the apparatus, the apparatus can preferably further comprise means for selectively energizing any one of the plurality of microunits.

The apparatuses of the present invention can be designed to generate any type of desired physical force(s), and preferably at least two different types of physical forces. In one specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces selected from the group consisting of electric, magnetic, acoustic, mechanical and optical forces. In another specific embodiment, the internal structures of the apparatuses are capable of generating at least two

different types of physical forces selected from the group consisting of electric, magnetic, acoustic, and optical forces. In still another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, optical and mechanical forces. In still another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces selected from the group consisting of electric, acoustic, optical, and mechanical forces. In still another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces selected from the group consisting of electric, magnetic, acoustic forces, and mechanical forces. In still another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces selected from the group consisting of magnetic, acoustic, optical, and mechanical forces. In yet another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces that do not include the combination of optical force and a dielectrophoretic force. In yet another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces that do not include the combination of acoustic forces generated by a standing wave acoustic field and an electrostatic force generated by a uniform DC field. In yet another specific embodiment, the internal structures of the apparatuses are capable of generating at least two different types of physical forces that do not include the combination of mechanical forces and electric forces.

There are a number of approaches for constructing apparatuses to generate different types of physical forces.

Electric forces may be generated by using any internal electrode structures in an apparatus, which are energized by electrical signal sources. The internal structures for generating electric forces may be located on a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Examples of the internal structures located on substrates for generating electric forces include all the "built-in" electrode structures on substrates, as described above for generating electric fields, as long as the apparatuses comprise the said substrates. Examples of those built-in electrodes structures include, but are not limited to, the following: planner electrodes covering the whole surface of the substrate or a large surface area of the substrate ("large" means, in a single dimension, at least ten times larger than the size of moiety to be manipulated), interdigitated castellated

electrodes, parallel electrodes, spiral electrodes, comb type electrodes, polynomial electrodes, individually addressable electrode array with electrode elements being circle, square, diamond or many other regular or irregular shapes.

The internal structures for generating electric forces may be located off or outside the substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Such examples include, but not limited to, electrode elements formed by using thin electric-conducting wires (e.g., 10 micron to 2000 micron in diameter). These wires may be wrapped around a substrate, or may be located at specific positions close to a substrate. The electric wires are structurally linked to the substrate of an apparatus comprising the substrate and are internal structures of the apparatus. When electrical signals are connected to these wires, electrical fields are produced in the region surrounding the wires, including the regions on or close to the substrates. Thus, when moieties of appropriate properties are introduced, electric forces are exerted on the moieties. Electric forces may be divided into electrostatic forces, conventional dielectrophoretic forces, and traveling-wave dielectrophoretic forces. The detailed description of these forces will be provided later.

Magnetic forces may be generated by using any internal structures in an apparatus, which are capable of generating magnetic forces when used in combination with external energy sources. The internal structures for generating magnetic forces may be located on a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Examples of the internal structures located on substrates for generating magnetic forces include all the "built-in" structures on substrates, as described above for generating magnetic fields, as long as the apparatuses comprise the said substrates. Examples of those "built-in" structures include, but are not limited to, the electromagnetic unit arrays as disclosed in the co-pending U.S. Patent Application Serial No. 09/399, 299, filed September 16, 1999, which is incorporated by reference in its entirety.

The internal structures for generating magnetic forces may be located off or outside the substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Such examples include, but are not limited to, electromagnetic elements formed by using wrapping electric-conducting wires (e.g., 10 micron to 2000 micron in diameter) around a magnetic core (e.g. made of ferromagnetic material). The electromagnetic elements may then be positioned at specific positions close to a substrate. The electromagnetic units are structurally linked to the substrate of an apparatus that

comprise the substrate. The electromagnetic elements are internal structures of the apparatus. When electrical currents from external signal sources are applied to the electromagnetic elements, magnetic fields are produced in the region surrounding the electromagnetic elements, including the regions on or close to the substrates. Thus, when moieties having certain magnetic properties are introduced, magnetic forces are exerted on the moieties. The detailed description of the magnetic forces will be provided later.

Acoustic forces may be generated by using any internal structures in an apparatus, which are capable of generating acoustic forces when used in combination with external energy sources. The internal structures for generating acoustic forces may be located on a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Examples of the internal structures located on substrates for generating acoustic forces include all the "built-in" structures on substrates, as described above for generating acoustic fields, as long as the apparatuses comprise the said substrates. Examples of those "built-in" structures include, but are not limited to, the cases where piezoelectric ceramic discs are used as substrates and the surfaces of the ceramics are deposited and processed with electrode arrays of different geometries.

The internal structures for generating acoustic forces may be located off or outside the substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Such examples include, but not are limited to, an acoustic wave source formed by using piezoelectric materials or formed with other approaches. The acoustic wave source may be positioned at specific positions close to a substrate. The acoustic wave source is structurally linked to the substrate of an apparatus that comprise the substrate. The acoustic wave source is an internal structure of the apparatus. When external signals (e.g. electrical voltages from an electrical voltage source) are applied to the acoustic wave source, acoustic fields are produced in the region surrounding the acoustic wave sources, including the regions on or close to the substrates. Thus, when moieties having certain properties are introduced, acoustic forces are exerted on the moieties. The detailed description of the acoustic forces will be provided later.

Optical forces may be generated by using any internal structures in an apparatus, which are capable of generating optical forces when used in combination with external energy sources. The internal structures for generating optical forces may be located on a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Examples of the internal structures located on substrates for generating

optical forces include all the "built-in" structures on substrates, as described above for generating optical fields, as long as the apparatuses comprise the said substrates. Examples of those "built-in" structures include, but are not limited to, the optical elements and arrays that are incorporated on a substrate, and the electro-optical elements and arrays that are incorporated on a substrate.

The internal structures for generating optical forces may be located off or outside the substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Such examples include, but are not limited to, an optical device that comprise an array of optical elements such as filters, lenses on an optical substrate. The optical device may be positioned at specific positions close to a substrate. The optical device is structurally linked to the substrate of an apparatus that comprise the substrate. The optical device is an internal structure of the apparatus. When external optical signals are applied to the optical device, optical fields are produced in the region close to the optical device, including the regions on or close to the substrates. Thus, when moieties having certain properties are introduced, optical forces can be exerted on the moieties. The detailed description of the optical forces will be provided later.

Mechanical forces may be generated by using any internal structures in an apparatus, which are capable of generating mechanical forces when used in combination with external energy sources. The internal structures for generating mechanical forces may be located on a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Examples of the internal structures located on substrates for generating mechanical forces include all the "built-in" structures on substrates, as described above for generating velocity fields in the medium that is in direct contact with or is in indirect contact with the substrates, as long as the apparatuses comprise the said substrates. Examples of those "built-in" structures include, but are not limited to, different types of the electro-mechanical elements that are incorporated on a substrate, heating and/or cooling elements that are incorporated on a substrate, specific surface configurations on a substrate.

The internal structures for generating mechanical forces may be located off or outside the substrate, which is part of the apparatus and holds or supports the moiety to be manipulated. Such examples include, but are not limited to, an electro-mechanical device such as valves, pumps. The electro-mechanical device is positioned at specific locations with respect to a substrate and is structurally linked to the substrate of an apparatus that comprise the substrate. The mechanical device is an internal structure of the apparatus.

When external electrical signals are applied to the electro-mechanical device, certain motions of the electro-mechanical device, or certain motions of the components within the electro-mechanical devices will occur, causing the medium that is in direct contact with, or is indirect contact with the electro-mechanical devices to move. The motion of medium results in a velocity filed. Thus, when moieties having certain properties are introduced, mechanical forces can be exerted on the moieties. The detailed description of the mechanical forces will be provided later.

Other types of physical forces may be generated by using any internal structures in an apparatus, which are capable of generating such physical forces when used in combination with external energy sources. Depending on the nature of such forces and depending on the apparatus configuration, the internal structures for generating such physical forces may be located on a substrate or off/outside a substrate, which is part of the apparatus and holds or supports the moiety to be manipulated.

The scale of the internal structures for generating desired physical forces must be compatible with and useable in microfluidic applications. For example, the internal structures can be micro-scale structures. Normally, the micro-scale structures have characteristic dimension of basic structural elements in the range from about 0.1 micron to about 20 mm scale. Preferably, the micro-scale structures have characteristic dimension of basic structural elements in the range from about 1 micron to about 1 mm scale.

The present apparatus can be used in a wide variety of applications in chemical/ biochemical/ biological reactions, processes and procedures. These reactions, processes and procedures may be used for biomedical research, clinical diagnosis and pharmaceutical drug development. The apparatus in the present invention may be used for many processes, such as manipulating moieties, facilitating chemical/ biochemical/ biological reactions, controlling chemical/ biochemical/ biological processes e.g. temperature control within a reaction chamber, for controlling fluidic flow. The internal structures or internal elements of the apparatuses may be designed to suit its particular uses. A variety of apparatuses having different internal structures may be constructed or built for different applications. In addition to the internal structures and/or elements described above for producing physical forces and the substrate for supporting or holding moiety or moieties to be manipulated, the apparatus may contain other structural elements that suit its particular uses. For example, if the apparatus is used for moiety manipulation, the apparatus may further comprise structural elements such as cover plates, spacers, gaskets, and input and

output ports so that the constructed apparatus is suitable for introducing, holding and removing a moiety or moieties to be manipulated to, in and out of the apparatus. The substrate that holds moiety to be manipulated may further comprise a functional layer on the support for immobilizing a moiety on the support.

C. Manipulation methods

The present chips and apparatuses can be used for manipulating any types of moieties when the moieties are involved in certain processes, such as physical, chemical, biological, biophysical or biochemical processes, etc., in a chip format. Moieties to be manipulated can be cells, cellular organelles, viruses, molecules or an aggregate or complex thereof. Moieties to be manipulated can be pure substances or can exist in a mixture of substances wherein the target moiety is only one of the substances in the mixture. For example, cancer cells in the blood from leukemia patients and cancer cells in the solid tissues from patients with solid tumors can be the moieties to be manipulated. Similarly, various blood cells such as red and white blood cells in the blood can be the moieties to be manipulated.

In one aspect, the present invention is directed to a method for manipulating a moiety, which method comprises: a) introducing a moiety to be manipulated into an apparatus comprising a substrate and at least two different types of internal structures, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical force; and b) allowing the internal structures of said apparatus, in combination of an external energy source, to exert at least two different types of physical forces on said moiety, whereby said moiety is manipulated by said physical forces.

The "at least two different types of physical forces" can be exerted on the moiety sequentially or simultaneously. The "at least two different types of physical forces" can be exerted on a single moiety sequentially or simultaneously. For example, an apparatus capable of producing acoustic forces and conventional dielectrophoretic forces may be used to exert these two types of forces simultaneously on moieties such as cells, or microparticles. Alternatively, the apparatus may be operated in the following procedure. First, the acoustic force generating elements are turned on so that moieties (e.g. cells, microparticles) experience acoustic forces for a specified length of time. Secondly, the acoustic force generating elements are turned off, and then the conventional

dielectrophoretic force generating elements are turned on so that the moieties (e.g. cells, microparticles) experience conventional dielectrophoretic forces.

The "at least two different types of physical forces" can be exerted on a plurality of moieties sequentially or simultaneously. For example, an apparatus capable of producing acoustic forces and conventional dielectrophoretic forces may be used to exert these two types of forces simultaneously on two types of moieties such as cells and microbeads. Thus, both types of moieties experience acoustic forces and conventional dielectrophoretic forces. In another example, an apparatus capable of producing magnetic forces and traveling wave dielectrophoretic forces may be used to exert these two types of forces simultaneously, and respectively, on two types of moieties such as magnetic beads and certain types of biological cells. Thus, magnetic forces are exerted only on magnetic microbeads and traveling wave dielectrophoretic forces may be exerted only on biological cells. In still another example, an apparatus capable of producing magnetic forces and traveling wave dielectrophoretic forces may be operated in the following procedure. First, the magnetic force generating elements are turned on so that magnetic microbeads experience magnetic forces for a specified length of time. Secondly, the magnetic force generating elements are turned off, and then the traveling wave dielectrophoretic force generating elements are turned on so that the biological cells experience traveling-wave dielectrophoretic forces.

Although the present method can be used to manipulate a single moiety at a time, the present method is preferably used to manipulate a plurality of moieties simultaneously. In some cases, the moiety to be manipulated can be contained in a mixture and the moiety is selectively manipulated. Selective manipulation refers to the manipulation process that the moiety that is being manipulated is selectively processed, and/or is separated from the mixture, and/or is caused to experience different manipulation forces or manipulation procedures from other moieties or other particles or other molecules in the mixture. In other cases, the moiety to be manipulated constitutes a mixture and the entire mixture is manipulated.

The moieties to be manipulated include the ones that can be manipulated directly by various physical forces and the ones that cannot be manipulated directly by various physical forces and have to be manipulated through the manipulation of the binding partner-moiety complex. In specific embodiments, moieties to be manipulated are cells, cellular organelles, viruses, molecules or an aggregate or complex thereof.

The present methods can be used for any type of manipulations. Non-limiting examples of the manipulations include transportation, focusing, enrichment, concentration, aggregation, trapping, repulsion, levitation, separation, fractionation, isolation or linear or other directed motion of the moieties.

The apparatus used in the present method must contain two or more different types of structures internal to said apparatus, each of which is capable of, in combination of an external energy source, exerting one type of physical forces on said moiety. Any physical forces can be used in the present method. In one specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of electric, (*e.g.*, electrostatic, conventional dielectrophoretic traveling wave dielectrophoretic), magnetic, acoustic, optical, and mechanical forces. In another specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of electric (*e.g.*, electrostatic, conventional dielectrophoretic and traveling wave dielectrophoretic), magnetic, acoustic, optical forces. In still another specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of electric (*e.g.*, electrostatic, conventional dielectrophoretic and traveling wave dielectrophoretic), magnetic, acoustic and mechanical forces. In still another specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of electric (*e.g.*, electrostatic, conventional dielectrophoretic and traveling wave dielectrophoretic), magnetic, optical and mechanical forces. In still another specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of electric (*e.g.*, electrostatic, conventional dielectrophoretic and traveling wave dielectrophoretic), acoustic, optical and mechanical forces. In still another specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of physical forces selected from the group consisting of magnetic, acoustic, optical and mechanical forces.

In yet specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at least two different types of any physical

forces that do not include the combination of optical force and dielectrophoresis forces. In yet specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at last two different types of any physical forces that do not include the combination of acoustic force produced by a standing-wave acoustic field, and electrostatic force produced by a uniform electric field. In yet specific embodiment, the internal structures of the apparatus are capable of exerting, on the moiety to be manipulated, at last two different types of any physical forces that do not include the combination of mechanical forces and electric forces.

In another aspect, the present invention is directed to a method for manipulating a moiety, which method comprises: a) introducing a moiety to be manipulated onto a chip comprising a substrate and at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination of an external energy source, generating one type of physical field; and b) allowing the built-in structures of said chip, in combination of an external energy source, to exert at least two different types of physical forces on said moiety, whereby said moiety is manipulated by said physical forces.

Non-limiting examples of manipulatable cells include animal, plant, fungi, bacteria, recombinant or cultured cells. Animal, plant, fungus, bacterium cells to be manipulated can be derived from any genus or subgenus of the Animalia, Plantae, fungus or bacterium kingdom. Cells derived from any genus or subgenus of ciliates, cellular slime molds, flagellates and microsporidia can also be manipulated. Cells derived from birds such as chickens, vertebrates such as fish and mammals such as mice, rats, rabbits, cats, dogs, pigs, cows, ox, sheep, goats, horses, monkeys and other non-human primates, and humans can be manipulated by the present chips, apparatuses and methods.

For animal cells, cells derived from a particular tissue or organ can be manipulated. For example, connective, epithelium, muscle or nerve tissue cells can be manipulated. Similarly, cells derived from an accessory organ of the eye, annulospiral organ, auditory organ, Chievitz organ, circumventricular organ, Corti organ, critical organ, enamel organ, end organ, external female gential organ, external male genital organ, floating organ, flower-spray organ of Ruffini, genital organ, Golgi tendon organ, gustatory organ, organ of hearing, internal female genital organ, internal male genital organ, intromittent organ, Jacobson organ, neurohemal organ, neurotendinous organ, olfactory organ, otolithic organ, ptotic organ, organ of Rosenmüller, sense organ, organ of smell, spiral organ,

subcommissural organ, subfornical organ, supernumerary organ, tactile organ, target organ, organ of taste, organ of touch, urinary organ, vascular organ of lamina terminalis, vestibular organ, vestibulocochlear organ, vestigial organ, organ of vision, visual organ, vomeronasal organ, wandering organ, Weber organ and organ of Zuckerkandl can be manipulated. Preferably, cells derived from an internal animal organ such as brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, gland, internal blood vessels, etc can be manipulated. Further, cells derived from any plants, fungi such as yeasts, bacteria such as eubacteria or archaebacteria can be manipulated. Recombinant cells derived from any eucaryotic or prokaryotic sources such as animal, plant, fungus or bacterium cells can also be manipulated. Body fluid such as blood, urine, saliva, bone marrow, sperm or other ascitic fluids, and subfractions thereof, *e.g.*, serum or plasma, can also be manipulated.

Manipulatable cellular organelles include nucleus, mitochondria, chloroplasts, ribosomes, ERs, Golgi apparatuses, lysosomes, proteasomes, secretory vesicles, vacuoles or microsomes. Manipulatable viruses, whether intact viruses or any viral structures, *e.g.*, viral particles, in the virus life cycle can be derived from viruses such as Class I viruses, Class II viruses, Class III viruses, Class IV viruses, Class V viruses or Class VI viruses.

Manipulatable intracellular moiety include any moiety that resides or is otherwise located within a cell, *i.e.*, located in the cytoplasm or matrix of cellular organelle; attached to any intracellular membrane; resides or is otherwise located within periplasma, if there is one; or resides or is otherwise located on cell surface, *i.e.*, attached on the outer surface of cytoplasm membrane or cell wall, if there is one. Any desired intracellular moiety can be isolated from the target cell(s). For example, cellular organelles, molecules or an aggregate or complex thereof can be isolated. Non-limiting examples of such cellular organelles include nucleus, mitochondria, chloroplasts, ribosomes, ERs, Golgi apparatuses, lysosomes, proteasomes, secretory vesicles, vacuoles or microsomes, membrane receptors, antigens, enzymes and proteins in cytoplasm.

Manipulatable molecules can be inorganic molecules such as ions, organic molecules or a complex thereof. Non-limiting examples of manipulatable ions include sodium, potassium, magnesium, calcium, chlorine, iron, copper, zinc, manganese, cobalt, iodine, molybdenum, vanadium, nickel, chromium, fluorine, silicon, tin, boron or arsenic ions. Non-limiting examples of manipulatable organic molecules include amino acids,

peptides, proteins, nucleosides, nucleotides, oligonucleotides, nucleic acids, vitamins, monosaccharides, oligosaccharides, carbohydrates, lipids or a complex thereof.

Any amino acids can be manipulated by the present chips, apparatuses and methods. For example, a D- and a L-amino-acid can be manipulated. In addition, any building blocks of naturally occurring peptides and proteins including Ala (A), Arg (R), Asn (N), Asp (D), Cys (C), Gln (Q), Glu (E), Gly (G), His (H), Ile (I), Leu (L), Lys (K), Met (M), Phe (F), Pro (P) Ser (S), Thr (T), Trp (W), Tyr (Y) and Val (V) can be manipulated.

Any proteins or peptides can be manipulated by the present chips, apparatuses and methods. For example, enzymes, transport proteins such as ion channels and pumps, nutrient or storage proteins, contractile or motile proteins such as actins and myosins, structural proteins, defense protein or regulatory proteins such as antibodies, hormones and growth factors can be manipulated. Proteineous or peptidic antigens can also be manipulated.

Any nucleic acids, including single-, double and triple-stranded nucleic acids, can be manipulated by the present chips, apparatuses and methods. Examples of such nucleic acids include DNA, such as A-, B- or Z-form DNA, and RNA such as mRNA, tRNA and rRNA.

Any nucleosides can be manipulated by the present chips, apparatuses and methods. Examples of such nucleosides include adenosine, guanosine, cytidine, thymidine and uridine. Any nucleotides can be manipulated by the present methods. Examples of such nucleotides include AMP, GMP, CMP, UMP, ADP, GDP, CDP, UDP, ATP, GTP, CTP, UTP, dAMP, dGMP, dCMP, dTMP, dADP, dGDP, dCDP, dTDP, dATP, dGTP, dCTP and dTTP.

Any vitamins can be manipulated by the present chips, apparatuses and methods. For example, water-soluble vitamins such as thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folate, vitamin B_{12} and ascorbic acid can be manipulated. Similarly, fat-soluble vitamins such as vitamin A, vitamin D, vitamin E, and vitamin K can be manipulated.

Any monosaccharides, whether D- or L-monosaccharides and whether aldoses or ketoses, can be manipulated by the present chips, apparatuses and methods. Examples of monosaccharides include triose such as glyceraldehyde, tetroses such as erythrose and threose, pentoses such as ribose, arabinose, xylose, lyxose and ribulose, hexoses such as

allose, altrose, glucose, mannose, gulose, idose, galactose, talose and fructose and heptose such as sedoheptulose.

Any lipids can be manipulated by the present chips, apparatuses and methods. Examples of lipids include triacylglycerols such as tristearin, tripalmitin and triolein, waxes, phosphoglycerides such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and cardiolipin, sphingolipids such as sphingomyelin, cerebrosides and gangliosides, sterols such as cholesterol and stigmasterol and sterol fatty acid esters. The fatty acids can be saturated fatty acids such as lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid and lignoceric acid, or can be unsaturated fatty acids such as palmitoleic acid, oleic acid, linoleic acid, linolenic acid and arachidonic acid.

For any moieties that cannot be directly manipulated with the desired physical forces, binding partners that themselves can be directly manipulated with the desired physical forces can be coupled to the moieties and the manipulation of such moieties can be effected through the manipulation of coupled binding partner-moiety complexes. Any binding partners that both bind to the moieties with desired affinity or specificity and are manipulatable with the compatible physical force(s) can be used in the present methods. The binding partners can be cells such as animal, plant, fungus or bacterium cells, cellular organelles such as nucleus, mitochondria, chloroplasts, ribosomes, ERs, Golgi apparatuses, lysosomes, proteasomes, secretory vesicles, vacuoles or microsomes; viruses, microparticles or an aggregate or complex thereof. The cells, cellular organelles and viruses described in this Section C can also be used as binding partners.

The microparticles used in the methods have a dimension from about 0.01 micron to about ten centimeters. Preferably, the microparticles used in the present method have a dimension from about 0.01 micron to about several thousand microns. Also preferably, the microparticles used are plastic particles, polystyrene microbeads, glass beads, magnetic beads or hollow glass spheres, particles of complex compositions, microfabricated free-standing microstructures.

The moiety to be manipulated can be coupled to the surface of the binding partner with any methods known in the art. For example, the moiety can be coupled to the surface of the binding partner directly or via a linker, preferably, a cleavable linker. The moiety can also be coupled to the surface of the binding partner via a covalent or a non-covalent linkage. Additionally, the moiety can be coupled to the surface of the binding partner via a

specific or a non-specific binding. Preferably, the linkage between the moiety and the surface of the binding partner is a cleavable linkage, *e.g.*, a linkage that is cleavable by a chemical, physical or an enzymatic treatment. Also preferably, the methods for coupling and/or decoupling the moieties to their binding partners disclosed in the co-pending U.S. Application entitled "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US application no. 09/636,104) by Wang et al, filed on August 10, 2000 can be used. Preferably, the moiety to be manipulated is substantially coupled onto surface of the binding partner. More preferably, the moiety to be manipulated is completely coupled onto surface of the binding partner.

Preferably, the methods for manipulating the moieties through the use of binding partners disclosed in the co-pending U.S. Application entitled "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US application no. 09/636,104) by Wang et al, filed on August 10, 2000 can be used for manipulating moieties that cannot be directly manipulated with the desired physical forces.

The above-described methods can also be used in manipulating a moiety in a non-chip format or without using the above-described apparatuses. Accordingly, in yet another aspect, the present invention is directed to a method for manipulating a moiety, which method comprises exerting at least two different types of physical forces on a moiety, whereby said moiety is manipulated by said physical forces. The physical forces can be selected from the forces including magnetic forces, dielectrophoretic forces, acoustic forces, optical forces and mechanical forces. For example, combination of magnetic force with dielectrophoretic force may be used for manipulating moieties. In another example, combination of magnetic force with acoustic force may be used for manipulating moieties.

The moiety can be manipulated in a liquid, or gaseous state/medium, or a combination thereof. Preferably, the moiety is manipulated in a liquid medium. The liquid medium can be a suspension, a solution or a combination thereof. The liquid medium can be contained and the moiety can be manipulated in any liquid container. Preferably, the liquid container used is the one(s) generally used in laboratory settings such as a fluidic chamber, beaker, a flask, a cylinder, a tube, *e.g.*, a test tube, an Eppendorf tube, a centrifugation tube or a collection tube, *etc.*, a dish, *e.g.*, a culture dish or a petridish, and a multiwell plate, *e.g.*, a 96-well, 384-well, 480-well, or 960-well plate, etc. The structures that are needed for exerting the physical forces on the moiety(ies) can be separated from the liquid containers or can be linked or attached to the liquid containers.

The structures for generating magnetic forces may take the form of electromagnetic coils that can be energized to produce the magnetic fields using electrical current sources. The electromagnetic coils can be separated from the liquid containers. And in operation, the electromagnetic coils may be placed in the vicinity of the liquid containers so that the magnetic fields produced by the energized magnetic coils can be coupled into the liquid containers. The magnetic particles in the liquid containers can thus be manipulated by such magnetic fields. Alternatively, the electromagnetic coils can be linked to the liquid containers. Another approach for producing the magnetic fields involves the use of permanent magnets. Again, permanent magnets can be separated from the liquid containers or can be linked to the liquid containers. Other approaches that can generate sufficient magnetic fields in the liquid containers may also be used.

The structures for producing dielectrophoresis forces may involve electric wires and/or other electric conductors. These electric wires or conductors have specific geometrical relationships with each other. The wires and/or other conductors can be separated from the liquid containers. And in operation, the wires and/or other conductors may be placed in the vicinity of the liquid contains so that the AC electrical fields produced by the energized electric wires and/or other conductors can be coupled into the liquid containers. The moieties in the liquid containers can thus be manipulated by such electric fields. Alternatively, the electric wires and/or other conductors can be linked to the liquid containers. Other approaches that can generate sufficient electric fields in the liquid containers may also be used.

The structures for producing acoustic forces may involve certain acoustic or ultrasonic wave sources. Such acoustic or ultrasonic wave sources may have piezoelectric elements that can be energized by electrical fields to produce acoustic or ultrasonic waves. The wave sources can be separated from the liquid containers. And in operation, the acoustic or ultrasonic wave sources may be placed in the vicinity of the liquid contains so that the acoustic or ultrasonic waves produced by the wave sources (that have been energized) can be coupled into the liquid containers. The moieties in the liquid containers can thus be manipulated by such acoustic or ultrasonic wave fields. Alternatively, the acoustic or ultrasonic wave fields can be linked to the liquid containers.

The structures for producing optical forces may involve certain optical sources and optical focusing devices/apparatuses. The optical focusing devices/apparatuses may involve optical lenses, optical filters or other optical elements that can produce optical

fields in the liquid containers when the liquid containers are placed with specific structural relationships with the optical focusing devices/apparatuses. The moieties in the liquid containers can thus be manipulated by such optical fields.

The structures for producing mechanical forces may involve certain fluidic pumps and valves. Such pumps and valves may produce a fluidic flow in the liquid containers. Mechanical forces will be exerted on the moieties due to such fluidic flow in the liquid containers. The particles are thus manipulated.

In one embodiment, the method for manipulating moiety utilizes at least two different types of physical forces that are exerted on the moiety sequentially. In another embodiment, the method for manipulating moiety is utilizing at least two different types of physical forces that are exerted on the moiety simultaneously.

In certain embodiments, a plurality of moieties are manipulated simultaneously by utilizing the manipulation method described above. For example, a plurality of moieties are manipulated simultaneously through use of more than one type of physical forces so that at least two different moieties are manipulated by different types of physical forces. In some other embodiments, a plurality of moieties are manipulated sequentially. For example, a plurality of moieties are manipulated sequentially through use of more than one type of physical forces so that at least two different moieties are manipulated by different types of physical forces.

In other embodiments, the moiety to be manipulated is contained in a mixture and the moiety is selectively manipulated using the manipulation method described above. In yet other embodiments, the moiety to be manipulated constitutes a mixture and the entire mixture is manipulated.

The present manipulation method is applicable to any moiety type such as a cell, a cellular organelle, a virus, a molecule and an aggregate or complex thereof. The cell may be an animal cell, a plant cell, a fungus cell, a bacterium cell, a recombinant cell or a cultured cell. The cellular organelle may be a nuclei, a mitochondrion, a chloroplast, a ribosome, an ER, a Golgi apparatus, a lysosome, a proteasome, a secretory vesicle, a vacuole or a microsome. The molecule may be an inorganic molecule, an organic molecule or a complex thereof. The inorganic molecule may be an ion such as a sodium, a potassium, a magnesium, a calcium, a chlorine, an iron, a copper, a zinc, a manganese, a cobalt, an iodine, a molybdenum, a vanadium, a nickel, a chromium, a fluorine, a silicon, a tin, a boron or an arsenic ion. The organic molecule may be an amino acid, a peptide, a

protein, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a vitamin, a monosaccharide, an oligosaccharide, a carbohydrate, a lipid or a complex thereof.

The present manipulation method can be utilized in transporting, focusing, enriching, concentrating, aggregating, trapping, repulsing, levitating, separating, fractionating, isolating or directing linear or other directed motion of the moiety.

D. Physical fields and forces

Exemplary physical fields and physical forces, and exemplary structures for generating such fields are further described in the following subsections.

(I) Acoustic forces

Acoustic force refers to the force that is generated on moieties, *e.g.*, particles and/or molecules, by an acoustic wave field. It may also be termed acoustic radiation forces. The acoustic forces can be used for manipulating, *e.g.*, trapping, moving, directing, handling, particles in fluid. The use of the acoustic force in a standing ultrasound wave for particle manipulation has been demonstrated for concentrating erythrocytes (Yasuda et al, *J. Acoust. Soc. Am.*, 102(1):642-645 (1997)), focusing micron-size polystyrene beads (0.3 to 10 micron in diameter, Yasuda and Kamakura, *Appl. Phys. Lett*, 71(13):1771-1773 (1997)), concentrating DNA molecules (Yasuda et al, *J. Acoust. Soc. Am.*, 99(2):1248-1251, (1996)), batch and semicontinuous aggregation and sedimentation of cells (Pui et al, *Biotechnol. Prog.*, 11:146-152 (1995)). By competing electrostatic and acoustic radiation forces, separation of polystyrene beads of different size and charges have been reported (Yasuda et al, *J. Acoust. Soc. Am.*, 99(4):1965-1970 (1996); and Yasuda et al., *Jpn. J. Appl. Phys.*, 35(1):3295-3299 (1996)). Furthermore, little or no damage or harming effect was observed when acoustic radiation force was used to manipulate mammalian cells, as characterized in terms of ion leakage (for erythrocytes, Yasuda et al, *J. Acoust. Soc. Am.*, 102(1):642-645 (1997)) or antibody production (for hybridoma cells, Pui et al, *Biotechnol. Prog.*, 11:146-152 (1995)).

An acoustic wave can be established by an acoustic transducer, *e.g.*, piezoelectric ceramics such as PZT material. The piezoelectric transducers are made from "piezoelectric materials" that produce an electric field when exposed to a change in dimension caused by an imposed mechanical force (piezoelectric or generator effect). Conversely, an applied electric field will produce a mechanical stress (electrostrictive or motor effect) in the

materials. They transform energy from mechanical to electrical and vice-versa. When AC voltages are applied to the piezoelectric transducers, the vibration occurs to the transducers and such vibration can be coupled into a fluid that is placed in the chamber comprising the piezoelectric transducers.

For a multiple-force chip (MFC) comprising an acoustic transducer, a chamber may be constructed so that the chip forms a major surface of the chamber. When AC signals at appropriate frequencies are applied to the electrodes on the acoustic transducers, the alternating mechanical stress is produced within the piezoelectric materials and is transmitted into the liquid solutions in the chamber. Consider the situation where the chamber is set up so that a standing acoustic wave is established along the direction (e.g.: z-axis) of wave propagation and reflection, the standing wave spatially varying along the z axis in a fluid can be expressed as:

$$\Delta p(z) = p_0 \sin(kz)\cos(\omega t)$$

where Δp is acoustic pressure at z, p_0 is the acoustic pressure amplitude, k is the wave number ($2\pi / \lambda$, where λ is the wavelength), z is the distance from the pressure node, ω is the angular frequency, and t is the time. In one example, the standing-wave acoustic field may be generated by the superimposition of an acoustic wave generated from an acoustic transducer that forms a major surface of a chamber and the reflective wave from another major surface of the chamber that is positioned in parallel with the acoustic transducer and reflects the acoustic wave from the transducer. According to the theory developed by Yosioka and Kawasima (Acoustic Radiation Pressure on a Compressible Sphere by Yosioka K. and Kawasima Y. in Acustica, Volume 5, pages 167-173, 1955), the acoustic force $F_{acoustic}$ acting on a spherical particle in the stationary standing wave field is given by

$$F_{acoustic} = -\frac{4\pi}{3} r^3 k \; E_{acoustic} \; A \; \sin(2kz)$$

where r is the particle radius, $E_{acoustic}$ is the average acoustic energy density, A is a constant given by

$$A = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\gamma_p}{\gamma_m}$$

where ρ_m and ρ_p are the density of the particle and the medium, γ_m and γ_p are the compressibility of the particle and medium, respectively. The compressibility of a material

is the product of the density of the material and the velocity of acoustic-wave in the material. The compressibility is sometimes termed acoustic impedance. A is termed as the acoustic-polarization-factor.

When $A>0$, the particle moves towards the pressure node ($z=0$) of the standing wave.

When $A<0$, the particle moves away from the pressure node.

Clearly, the acoustic radiation forces acting on particles depend on acoustic energy density distribution and on particle density and compressibility. The particles having different density and compressibility will experience different acoustic-radiation-forces when they are placed into the same standing acoustic wave field. The acoustic radiation force acting on a particle of 10 micron in diameter can vary somewhere between < 0.01 and > 1000 pN, depending on the established acoustic energy density distribution.

The above analysis considers the acoustic radiation forces exerted on particles in a standing acoustic wave. Further analysis may be extended to the case of the acoustic radiation forces exerted on particles in a traveling-wave case. Generally, an acoustic wave field may consist of both standing and traveling-wave components. In such cases, particles in the chamber will experience acoustic radiation forces in the form other than those described by above equations. The following papers provide detailed analysis of acoustic radiation forces on spherical particles by traveling acoustic wave and standing acoustic waves: "Acoustic Radiation Pressure on a Compressible Sphere" by Yosioka K. and Kawasima Y. in Acustica, Volume 5, pages 167-173, 1955; and "Acoustic-Radiation force on a solid elastic sphere" by Hasegawa T. and Yosioka K. in Journal of Acoustic Society of America.

The acoustic radiation forces on particles may also be generated by various special cases of acoustic waves. For example, acoustic forces may be produced by a focused beam ("Acoustic radiation force on a small compressible sphere in a focused beam" by Wu and Du, *J. Acoust. Soc. Am.*, 87:997-1003 (1990)), or by acoustic tweezers ("Acoustic tweezers" by Wu *J. Acoust. Soc. Am.*, 89:2140-2143 (1991)).

Acoustic wave field established in a fluid can also induce a time-independent fluid flow, as termed acoustic streaming. Such fluid flow may also be utilized in biochip applications or microfluidic applications for transporting or pumping fluids. Furthermore, such acoustic-wave fluid flow may be exploited for manipulating molecules or particles in fluids. The acoustic streaming depends on acoustic field distributions and on fluid

properties ("Nonlinear phenomena" by Rooney J.A. in "Methods of Experimental Physics: Ultrasonics, Editor: P.D. Edmonds", Chapter 6.4, pages 319-327, Academic Press, 1981; "Acoustic Streaming" by Nyborg W.L.M. in "Physical Acoustics, Vol. II-Part B, Properties of Polymers and Nonlinear Acoustics, Chapter 11, pages 265-330).

Exemplary embodiments of acoustic-force-generation elements in a MFC (multiple-force chip) include, but are not limited to, the following.

(1) A MFC employs a substrate that is made of piezoelectric material. Electrodes are deposited on the two major surfaces of the piezoelectric substrate, acoustic waves may be generated from the MFC substrate.

(2) A MFC employs a substrate that is made of piezoelectric material. Electrode arrays are deposited on one or two major surfaces of the piezoelectric substrate, multiple acoustic waves are generated from the electrode-array-defined locations on the MFC substrate. Electrode arrays may comprise individually addressable electrode elements or all connected electrode elements. The acoustic array described in U.S. Patent No. 6,029,518 may be used for such purposes.

(3) A MFC employs a substrate that is not made of piezoelectric material. Nevertheless, piezoelectric thin films may be deposited on the substrate. Such thin films may cover whole substrate and act as one acoustic-wave generation source. Alternatively, the thin films may be patterned so that individually addressable acoustic wave source array or connected-together acoustic wave source array may be formed. The piezoelectric thin film may be deposited over a portion of the MFC or all surfaces of the MFC.

(4) A MFC may employs a substrate wherein a portion of which is made of piezoelectric materials. Electrodes may be deposited on that portion of the substrate to produce acoustic wave sources.

(5) An active chip or biochip capable of producing certain type of active forces on molecules or particles is fabricated using non-piezoelectric materials. Nevertheless, a piezoelectric transducer (a single acoustic wave source or an array of acoustic wave source) may be bound to the active biochip to form a MFC.

In all the above cases, it is possible to include further variations to the acoustic wave generation elements so the focused acoustic beams are utilized. For example, Acoustic lenses may be used for focusing the acoustic waves (*e.g.*, "Nozzleless droplet

formation with focused acoustic beams by Elrod et al, *J. Applied Physics*, 65:3441 – 3447 (1989)).

(II) Electric (or electrical) forces.

Electric (or electrical) force refers to the force that is generated on moieties (e.g. particles, cells, etc) by an electric field. Electric force is also sometimes called electrical force, electrokinetic force. Depending on the field configurations and properties of the moieties in the field, different types of electric forces can be exerted on moieties. One type of electric forces is the electrostatic or electrophoretic force, which refers to the force that is generated on charged moieties (e.g. molecules, cells, particles) by a DC electrical field or a low-frequency AC electric field (less than 1 kHz). The electrostatic force is sometimes called electrophoretic force. Another type of electric forces is dielectrophoretic force, which refers to the force that is generated on charged or neutral moieties by a non-uniform AC electric field. The electric field must have non-uniform distribution in the field magnitude or the phase values of the field components, and moieties must have different dielectric properties from those of the surrounding medium so that the moieties are electrically polarized in a field, in order to produce non-zero dielectrophoretic force on moieties. There are two types of dielectrophoretic forces, the first type being conventional dielectrophoretic force and the second type being traveling wave dielectrophoretic force. The detailed descriptions of these forces are provided later.

II.A Electrostatic (or electrophoretic) forces

Electrostatic (or electrophoretic) force refers to the force that is generated on charged moieties (e.g., charged molecules or particles) by a DC electrical field or a low-frequency AC electric field (less than 1 kHz). The electrostatic force arises from the electrical field interacting with the static charge on the moieties (e.g. particles, molecules or cells). The electrostatic force F_E on a particle in an applied electrical field $E_z\vec{a}_z$ is given by

$$F_E = Q_p E_z \vec{a}_z$$

where Q_p is the effective electric charge on the particle. The direction of the electrostatic force on the charged particle depends on the polarity of the particle charge as well as the applied-field direction.

The DC electrical field in a MFC-based chamber or MFC-based apparatus for manipulating moieties, *e.g.*, molecules or particles, can be established by applying DC electrical signals to electrode or microelectrode elements that are fabricated on the MFC (multiple-force chip) or incorporated in the MFC-based chamber or the MFC-based apparatus.

The exemplary embodiments of electrodes or microelectrodes that are fabricated on a MFC include, but are not limited to, the following.

(1) Individually addressable microelectrode element arrays may be used. Each element may be applied with appropriate DC electrical signals so that DC electrical field distribution may be established and adjusted for required electrostatic manipulation of molecules and particles. Various electrode dimensions/geometries or electrode array dimensions may be used.

(2) Microelectrode element arrays where all the elements or portion of the elements are connected together may be used. Various electrode element geometry/dimension and various electrode array dimensions may be used.

(3) If required, electrodes may cover the whole or portion of the overall MFC. An uniform or non-uniform DC electric field may be applied.

II.B Dielectrophoretic (DEP) forces

Dielectrophoretic forces refer to the forces that are generated on moieties (e.g. particles or molecules) due to non-uniform distribution of an AC electric field. The DEP force arises from the interaction between the electric field induced polarization charges and the non-uniform electric field. The polarization charge is induced on moieties because of the applied field and because of the difference in dielectric properties between particles and particle suspending medium.

A single harmonic electrical field $\vec{E}(t)$ can generally be expressed in the time-domain as

$$\vec{E}(t) = \sum_{\alpha=x;y;z} E_{\alpha 0}\cos(2\pi f t + \varphi_\alpha)\vec{a}_\alpha ,$$

where $\vec{a}_\alpha$ (α=x, y, z) are the unit vectors in a Cartisian coordinate, $E_{\alpha 0}$ and φ_α are the magnitude and phase of the three field components. When a cell is subjected to such a non-uniform electric field ($E_{\alpha 0}$ and/or φ_α vary with position), a net dielectrophoretic force is

exerted on the cell because of the electric interaction between the field and the field-induced dipole moment. The DEP force is given by Wang et al in an article "An unified theory of dielectrophoresis and travelling-wave dielectrophoresis by Wang et al., *J. Phys. D: Appl. Phys.*, Vol. 27, Pages:1571-1574 (1994)),

$$\vec{F} = 2\pi\varepsilon_m r^3\left(\mathrm{Re}(f_{CM})\nabla E_{rms}^2 + \mathrm{Im}(f_{CM})\left(E_{x0}^2\nabla\varphi_x + E_{y0}^2\nabla\varphi_y + E_{z0}^2\nabla\varphi_z\right)\right),$$

where r is the particle radius, ε_m is the dielectric permittivity of the particle suspending medium, E_{rms} is the field RMS magnitude. The factor $f_{CM} = \left(\varepsilon_p^* - \varepsilon_m^*\right)/\left(\varepsilon_p^* + 2\varepsilon_m^*\right)$ is the dielectric polarization factor (the so-called Clausius-Mossotti factor). The complex permittivity is defined as $\varepsilon_x^* = \varepsilon_x - j\sigma_x/(2\pi f)$. The dielectric polarization factor depends on the frequency f of the applied field, conductivity σ_x and permittivity ε_x of the particle (x=p) and its suspending medium (x=m).

Thus, the DEP force generally has two components, *i.e.*, conventional DEP force and traveling-wave DEP force. The conventional DEP forces are associated with the in-phase component part of the field-induced polarization (reflected by the term $\mathrm{Re}(f_{CM})$, the real part of the factor f_{CM} interacting with the gradient of the field magnitude (∇E_{rms}^2). The term $\mathrm{Re}(f_{CM})$ is sometimes called conventional DEP factor. The traveling-wave DEP forces are associated with the out-of-phase component of the field-induced polarization (reflected by the term $\mathrm{Im}(f_{CM})$, the imaginary part of the factor f_{CM}) interacting with the gradient of the field phases ($\nabla\varphi_x, \nabla\varphi_y$ and $\nabla\varphi_z$). The term $\mathrm{Im}(f_{CM})$ is sometimes called traveling wave DEP factor.

(II.B.1) Conventional DEP (cDEP) force

Conventional DEP force refers to the force that is generated on moieties due to non-uniform distribution of the magnitude of an AC electric field. In the literature, the conventional DEP force is sometimes called simply DEP force. This simplification in terminology is avoided herein for clarity. As used in the literature papers "An unified theory of dielectrophoresis and travelling-wave dielectrophoresis by Wang et al. *J. Phys. D: Appl. Phys.* 27:1571-1574, (1994))" and "Non-uniform spatial distributions of both the magnitude and phase of AC electric fields determine dielectrophoretic forces by Wang et al., *Biochim Biophys Acta*, Vol. 1243, Pages: 185-194 (1995), "DEP force" is used herein

as a general term including both conventional DEP and traveling-wave DEP components. Conventional DEP force refers to the force that is generated on moieties due to non-uniform distribution of the magnitude of an AC electric field. Traveling-wave DEP force refers to the force that is generated on moieties due to a traveling-wave electric field (or due to non-uniform distribution of the phase values of field components).

The conventional DEP force $\vec{F}_{DEP}$ acting on a particle of radius r subjected to an electrical field of non-uniform magnitude is given by

$$\vec{F}_{DEPz} = 2\pi\varepsilon_m r^3 \chi_{DEP} \nabla E_{rms}^2$$

where E_{rms} is the RMS value of the field strength, ε_m is the dielectric permittivity of the medium. This equation for conventional DEP force is consistent with the general expression of DEP forces described above. The factor χ_{DEP} is the particle polarization factor, given by

$$\chi_{DEP} = \mathrm{Re}\left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right),$$

"Re" refers to the real part of the "complex number". The symbol $\varepsilon_x^* = \varepsilon_x - j\sigma_x/2\pi f$ is the complex permittivity (of the particle x=p, and the medium x=m). The parameters ε_p and σ_p are the effective permittivity and conductivity of the particle, respectively. These parameters may be frequency dependent. For example, a typical biological cell will have frequency dependent, effective conductivity and permittivity, at least, because of cytoplasm membrane polarization.

The above equation for the conventional DEP force can also be written as

$$\vec{F}_{DEP} = 2\pi\varepsilon_m r^3 \chi_{DEP}\, \mathrm{V}^2(\nabla\, \mathrm{p})$$

where p(x,y,z) is the square-field distribution for an unit-voltage excitation (*Voltage* V = 1 V) on the electrodes, V is the applied voltage.

Particles such as biological cells having different dielectric property (as defined by permittivity and conductivity) will experience different dielectrophoretic forces (both conventional DEP and traveling-wave DEP forces). For DEP manipulation of particles (including biological cells), conventional DEP forces acting on a particle of 10 micron in diameter can vary somewhere between 0.01 and 10000 pN.

A non-uniform electrical field can be established in a MFC-based chamber by applying AC signals to the microelectrodes incorporated on the MFC (multiple-force chip) structures. Various electrode element arrays and electrode arrays may be used. A number of DEP electrodes reported in the literature could be used, including, interdigitated castellated electrodes, polynomial electrodes, sinusoidal electrodes, pin-point electrodes, parallel electrodes, three-dimensional field cage electrodes. The following papers describe a number of electrode geometries, and all these electrodes could be utilized in a MFC structure, including "Dielectrophoretic Manipulation of Particles by Wang et al, in IEEE Transaction on Industry Applications, Vol. 33, No. 3, May/June, 1997, pages 660-669"; "Selective dielectrophoretic confinement of bioparticles in potential energy wells by Wang et al. *J. Phys. D: Appl. Phys.*, Vol. 26, pages:1278-1285"; "Positioning and manipulation of cells and microparticles using miniaturized electric field traps and traveling waves. By Fuhr et al., *Sensors and Materials.*, 7:131-146", "Non-uniform Spatial Distributions of Both the Magnitude and Phase of AC Electric Fields determine Dielectrophoretic Forces by Wang et al., *Biochim Biophys Acta*, Vol. 1243, pages:185-194 (1995)"; "Electrode design for negative dielectrophoresis, by Huang and Pethig, *Meas. Sci. Technol.*, Vol. 2, pages:1142-1146 (1991)"; "Positive and negative dielectrophoretic collection of colloidal particles using interdigitated castellated microelectrodes by Pethig et al., *J. Phys. D: Appl Phys.*, Vol. 25, pages: 881-888 (1992)"; "Three-dimensional electric field traps for manipulation of cells – calculation and experimental verification by Schnelle et al., *Biochim. Biophys. Acta.*, Vol. 1157, pages: 127-140 (1993)", "A 3-D microelectrode system for handling and caging single cells and particles, by Müller, et al., *Biosensors and Bioelectronics*, Vol. 14, pages: 247-256 (1999)"; "Dielectrophoretic field cages: technique for cell, virus and marcromolecule handling, by Fuhr et al., *Cellular Engineering.*, Autumn, pages 47-57 (1995)"; "Electrocasting – formation and structuring of suspended microbodies using A.C. generated field cages, by Fiedler S. et al., *Microsystem Technologies.*, Vol. 2, pages:1-7, (1995)"; "Dielectrophoretic sorting of particles and cells in a microsystem, by Fiedler et al., *Anal. Chem.*, Vol. 70, pages:1909-1915 (1998)".

Individually addressable microelectrode element arrays may be used. Each element may be applied with AC electrical signals so that non-uniform electrical field distribution may be established and adjusted for required dielectrophoretic manipulation of molecules and particles. Various electrode dimensions/geometries or electrode array dimensions may be used.

Microelectrode element arrays where all the elements or portion of the elements are connected together may be used. Various electrode element geometry, various electrode element dimension, various electrode array dimensions may be used.

Electrode elements may cover the whole or portion of the overall MFC.

(II.B.2) Traveling-wave dielectrophoretic (twDEP) forces.

Traveling-wave DEP force refers to the force that is generated on moieties (particles or molecules) due to a traveling-wave electric field. A traveling-wave electric field is characterized by the non-uniform distribution of the phase values of AC electric field components.

Here we analyze the traveling-wave DEP force for an ideal traveling-wave field. The dielectrophoretic force F_{twDEP} acting on a particle of radius r subjected to a traveling-wave electrical field $E = E\cos\left(2\pi(ft - z/\lambda_0)\right)\vec{a}_x$ (*i.e.*, a x-direction field is traveling along the z-direction) is given by

$$F_{twDEP} = -\frac{4\pi^2\varepsilon_m}{\lambda} r^3 \zeta_{TWD} E^2 \cdot \vec{a}_z$$

where E is the magnitude of the field strength, ε_m is the dielectric permittivity of the medium. ζ_{TWD} is the particle polarization factor, given by

$$\zeta_{twDEP} = \mathrm{Im}\left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right),$$

"Im" refers to the imaginary part of the "complex number". The symbol $\varepsilon_x^* = \varepsilon_x - j\sigma_x/2\pi f$ is the complex permittivity (of the particle x=p, and the medium x=m). The parameters ε_p and σ_p are the effective permittivity and conductivity of the particle, respectively. These parameters may be frequency dependent.

Particles such as biological cells having different dielectric property (as defined by permittivity and conductivity) will experience different dielectrophoretic forces. For traveling-wave DEP manipulation of particles (including biological cells), traveling-wave DEP forces acting on a particle of 10 micron in diameter can vary somewhere between 0.01 and 10000 pN.

A traveling wave electric field can be established in MFC-based chambers or MFC-based apparatuses by applying appropriate AC signals to the microelectrodes incorporated

on the MFC structures. For generating a traveling-wave-electric field, it is necessary to apply at least three types of electrical signals each having a different phase value. One approach to produce traveling wave electric field is to use four phase-quardrature signals (0, 90, 180 and 270 degrees) to energize four linear, parallel electrodes patterned on the chip surfaces. Such four electrodes form a basic, repeating unit. In most cases, there are at least two such units that are located next to each other. This will produce a traveling-electric field in the spaces above or near the electrodes. Another approach is to use four, parallel spiral electrode elements that are energized by four phase-quardrature signals (0, 90, 180 and 270 degrees), as described in "Dielectrophoretic manipulation of cells using spiral electrodes by Wang et al., *Biophys. J.*, Vol. 72, pages: 1887-1899 (1997)". As long as electrode elements are arranged following certain spatially sequential orders, applying phase-sequenced signals will result in establishing traveling electrical fields in the region close to the electrodes.

Exemplary embodiment of traveling-wave dielectrophoresis (twDEP) electrodes includes four parallel linear-spiral electrodes, parallel linear electrodes, spiral electrodes ("Dielectrophoretic manipulation of cells using spiral electrodes by Wang et al., *Biophys. J.*, Vol. 72, pages: 1887-1899 (1997)"; "Dielectrophoretic Manipulation of Particles by Wang et al, *IEEE Transaction on Industry Applications*, 33(3):660-669(1997)", "Electrokinetic behavior of colloidal particles in traveling electric fields: studies using yeast cells by Huang et al, *J. Phys. D: Appl. Phys.*, Vol. 26, pages: 1528-1535", "Positioning and manipulation of cells and microparticles using miniaturized electric field traps and traveling waves. By Fuhr et al., *Sensors and Materials.*, Vol. 7, pages:131-146", "Non-uniform spatial distributions of both the magnitude and phase of AC electric fields determine dielectrophoretic forces by Wang et al., *Biochim Biophys Acta.*, Vol. 1243, pages:185-194 (1995)".

Traveling-wave-dielectrophoresis forces have an unique feature in comparison with other types of physical force – it can transport particles in an one- or two-dimensional space (along a line or along certain trajectories on a plane surface). For example, the MFC may comprise multiple units (or reaction centers) and twDEP forces may be utilized to transport moieties (e.g. particles or molecule-particle complexes) between these units.

Traveling-wave-dielectrophoresis forces can also be used to control, switch, direct particles through particle switches or microparticle switches, as described in co-pending US applications, Attorney Docket No. Knobbe, Martens, Olson & Bear, LLP,

ARTLNCO.002a, entitled "APPARATUSES FOR SWITCHING AND MANIPULATING PARTICLES AND METHOD OF USE THEREOF" by Wang et al., filed on October 3, 2000. Various electrode structures that are disclosed in that application can be incorporated in the MFC and used for manipulating and controlling particles, moieties by traveling wave dielectrophoretic forces. For example, in one embodiment of microparticle switch, the particle switches comprises at least three sets of electrodes that are capable of producing respective traveling wave dielectrophoresis (twDEP) forces for the movement of particles along respective branches, wherein: said sets of electrodes are electrically independent from each other; and said branches are interconnected at a common junction to permit the twDEP forces to route particles from one of said branches to another of said branches.

(III) Magnetic forces.

Magnetic forces refer to the forces acting on a moiety, *e.g.*, particle due to the application of a magnetic field. In general, particles have to be magnetic or paramagnetic when sufficient magnetic forces are needed to manipulate particles. We consider the example of a typical magnetic particle made of super-paramagnetic material. When the particle is subjected to a magnetic field $\overline{B}$, a magnetic dipole $\overline{\mu}$ is induced in the particle

$$\begin{aligned}\overline{\mu} &= V_p(\chi_p - \chi_m)\frac{\overline{B}}{\mu_m},\\ &= V_p(\chi_p - \chi_m)\overline{H}_m\end{aligned}$$

where V_p is the particle volume, χ_p and χ_m are the volume susceptibility of the particle and its surrounding medium, μ_m is the magnetic permeability of medium, $\overline{H}_m$ is the magnetic field strength. The magnetic force $\overline{F}_{magnetic}$ acting on the particle is determined by the magnetic dipole moment and the magnetic field gradient:

$$\overline{F}_{magnetic} = -0.5\ V_p(\chi_p - \chi_m)\vec{H}_m \bullet \nabla\vec{B}_m,$$

where the symbols "$\bullet$" and "∇" refer to dot-product and gradient operations, respectively. Clearly, whether there is magnetic force acting on a particle depends on the difference in the volume susceptibility between the particle and its surrounding medium. Typically, particles are suspended in a liquid, non-magnetic medium (the volume susceptibility is close to zero) thus it is necessary to utilize magnetic particles (its volume susceptibility is

much larger than zero). The particle velocity $v_{particle}$ under the balance between magnetic force and viscous drag is given by:

$$v_{particle} = \frac{\overline{F}_{magnetic}}{6\pi r \eta_m}$$

where r is the particle radius and η_m is the viscosity of the surrounding medium. Thus to achieve sufficiently large magnetic manipulation force, the following factors should be considered: (1) the volume susceptibility of the magnetic particles should be maximized; (2) magnetic field strength should be maximized; and (3) magnetic field strength gradient should be maximized.

Magnetic fields can be established in MFC-based chambers by applying electric currents to microelectromagnetic elements. Each microelectromagnetic element is capable of producing magnetic field upon applying DC and/or ACelectric currents. An electromagnetic element may be an electric wire wrapped as a loop, or an electric coil wrapped around a magnetic core. A number of types of electromagnetic elements are described in the co-pending US Patent Application Serial Number 09/399,299, filed on September 16, 1999, which is incorporated by reference in its entirety. Those electromagnetic elements can be incorporated in the MFC. Other examples of electromagnetic units that can be incorporated on MFC include, but are not limited to, the following. Ahn, C., *et al.*, *J. Microelectromechanical Systems*. Volume 5: 151-158 (1996); Ahn, C., *et al.*, *IEEE Trans. Magnetics*. Volume 30: 73-79 (1994); Liakopoulos *et al.*, *in Transducers 97,* pages 485-488, presented in 1997 International Conference on Silid-State Sensors and Actuators, Chicago, June 16-19, 1997; US patent No. 5,883,760 by Naoshi *et al.*.

As an exemplary embodiment, the MFC may incorporate an array of individually addressable electromagnetic units. These units are positioned or structurally arranged in certain order so that when each of or some of or all of electromagnetic units are energized (=magnetized), desired magnetic field distributions can be established to produce magnetic forces acting on magnetic particles. In another example, the MFC may comprise multiple, interconnected electromagnetic units so that these units can be turned on or off in a synchronized order. Yet, in another example, the MFC may comprise only one electromagnetic unit that can be energized to produce magnetic fields.

Manipulation of magnetic particles includes the directed movement, focusing and trapping of magnetic particles. The motion of magnetic particles in a magnetic field is termed "magnetophoresis". Theories and practice of magnetophoresis for cell separation and other applications may be found in various literatures (*e.g.*, Magnetic Microspheres in Cell Separation, by Kronick, P. L. in Methods of Cell Separation, Volume 3, edited by N. Catsimpoolas, 1980, pages 115-139; Use of magnetic techniques for the isolation of cells, by Safarik I. And Safarikova M., in J. of Chromatography, 1999, Volume 722(B), pages 33-53; A fully integrated micromachined magnetic particle separator, by Ahn C. H. *et al.*, in J. of Microelectromechanical systems, 1996, Volume 5, pages 151-157).

(IV) Optical forces

Optical forces refer to the forces acting on moieties, *e.g.*, particles or molecules, due to a light intensity gradient. Optical forces are sometimes called optical radiation forces. When particles are placed into a light intensity field that has a non-uniform spatial distribution, a radiation force will be exerted on the particles. Such a radiation force depends on particle size, light intensity distribution, the refractive index of the particle and its surrounding medium. A number of literatures provide the details of light radiation forces ("Laser trapping in cell biology, by Wright *et al.*, in IEEE J. of Quantum Electronics, 1990, Volume 26, pages 2148-2157"; "Laser manipulation of atoms and particles, by Chu S. in Science, 1991, Volume 253, pages 861-866"). The optical forces are the so-called gradient-forces when a material (*e.g.*, a microparticle) with a refractive index different from that of the surrounding medium is placed in a light gradient. As light passes through polarizable material, it induces fluctuating dipoles. These dipoles interact with the electromagnetic field gradient, resulting in a force directed towards the brighter region of the light if the material has a refractive index larger than that of the surrounding medium. Conversely, an object with a refractive index lower than the surrounding medium experiences a force drawing it towards the darker region.

Optical forces or optical radiation forces have been exploited in "laser tweezers" that may be used to focus, trap, levitate and manipulate microparticles. The laser tweezers typically are single-beam gradient laser taps. The theory and practice of "laser tweezers" for various biological applications are described in various literatures (*e.g.*, "Making light work with optical tweezers, by Block S. M., in Nature, 1992, Volume 360, pages 493-496"; "Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime,

by Ashkin, A., in Biophys. J., 1992, Volume 61, pages 569-582"; "Laser trapping in cell biology, by Wright *et al.*, in IEEE J. of Quantum Electronics, 1990, Volume 26, pages 2148-2157"; "Laser manipulation of atoms and particles, by Chu S. in Science, 1991, Volume 253, pages 861-866").

To generate optical-radiation-force in a MFC-based chamber or an apparatus, the optical field distribution and/or light intensity distribution may be produced, for example, by the built-in optical elements and arrays on the MFC and the external optical signal sources, or by the built-in electro-optical elements and arrays on a MFC and the external structures are electrical signal sources. In the former case, when the light produced by the optical signal sources passes through the built-in optical elements and arrays, light is processed by these elements/arrays through, *e.g.*, reflection, focusing, interference, etc. Optical field distributions are generated in the regions around the MFC. In the latter case, when the electrical signals from the external electrical signal sources are applied to the built-in electro-optical elements and arrays, light is produced from these elements and arrays and optical fields are generated in the regions around the MFC. Other approaches may also be used to include built-in elements on the MFC to produce optical field distribution, resulting in optical forces.

Laser tweezers may be used to exert optical forces in an apparatus of the present invention. Various structures may be incorporated into the MFC or in the apparatus so that single or multiple laser tweezers may be introduced into the apparatus or MFC-based chamber. Single laser tweezers may be incorporated into the apparatus at critical locations where such laser tweezers are used for manipulating, attracting or repelling particles or molecules or molecule complexes. On the other hand, multiple laser tweezers may be positioned into the apparatus at a number of locations.

Optical forces or optical radiation forces may be used for positioning, controlling, manipulating particles or molecules. For example, particles may be trapped in a light intensity field defined by a laser tweezers. Or particles may be optically-guided or switched with an array of laser tweezers.

(V). Mechanical forces.

Mechanical forces refer to the forces acting on a moiety due to a velocity field in a medium in which the moiety is suspended, dissolved or placed. We consider the case of a spherical

particle of density ρ_p and diameter D_p is placed in a velocity field $\vec{u}_m$. The governing equation for particle velocity $\vec{u}_p$ is given by

$$\frac{d\vec{u}_p}{dt} = \frac{1}{\tau}(\vec{u}_m - \vec{u}_p) + \vec{f}_p,$$

where t is the time, $\vec{u}_m$ is the velocity of the medium, $\vec{f}_p$ is the combination of forces acting on the particle, including gravitation forces, and τ is the particle relaxation time, defined by

$$\tau = \frac{4\rho_p D_p^2}{3\mu_m C_D \mathrm{Re}_p},$$

where μ_m is the viscosity of the medium, C_D is the drag coefficient, Re_p is the particle Reynolds number defined by

$$\mathrm{Re}_p = \frac{|\vec{u}_m - \vec{u}_p| D_p \rho_m}{\mu_m},$$

where ρ_m is the density of the medium. The first term of the right hand side of the above equation is a generalization of the classical Stokes drag on a particle. It is well known that the Stokes drag force on a spherical particle is given by,

$$F_{Stokes\_Drag} = 3\pi D_p (\vec{u}_m - \vec{u}_p)\mu_m.$$

The Stokes drag force is exerted on the particle because of the relative velocity ($\vec{u}_m - \vec{u}_p$) of the medium with respect to the medium. Thus, when a particle (or a moiety of various types) is placed in a velocity field $\vec{u}_m$ of the medium, a mechanical force (the Stokes drag force) will be exerted on the particle to cause the particle to move in the same velocity as that of the medium at the position where the particle is located in the medium. Such a mechanical force may influence particle position, velocity, or other kinetic behavior, and may be used to manipulate particles.

The velocity field of a medium refers to the velocity distribution of a medium that is moving. The governing equation for the velocity of the medium is given by

$$\rho_0\left(\frac{\partial \vec{u}_m}{\partial t} + \vec{u}_m \bullet \nabla \vec{u}_m\right) = -\nabla p + \rho_0 \vec{f} + \mu_m \nabla^2 u_m - \rho_0 \vec{g}\left[\beta_T (T - T_0) + \sum_n \beta_{Cn} C_n\right]$$

where ρ_0 is the density of the medium at temperature T_0, $\vec{u}_m$ is the velocity of the medium, p is the pressure within the medium, $\vec{f}$ is the body force acting on the medium,

$\vec{g}$ is the gravitational acceleration force vector, β_T is the coefficient of volumetric expansion associated with the temperature variations, β_{Cn} is the volumetric expansion coefficient associated with the n'th species. The above parameters are all functions of the positions within the medium, except the density ρ_0 and gravitational acceleration force vector $\vec{g}$.

According to the above equation, there are several ways to cause a change in the velocity field of the medium. These are: (1) altering the pressure within the medium, (2) applying body force on the medium, (3) causing a temperature change or temperature gradient in the medium, (4) causing a change in the concentrations of species in the medium. Here we analyze several approaches to produce a velocity field, thus to produce mechanical forces on moieties in the medium.

(V.1) Mechanical forces caused by thermal convection

A temperature gradient may be generated in the medium. According to above equation, such a temperature gradient will result in the motion in the medium and lead to a velocity field within the medium. The motion of the medium results partially from the thermal diffusion in the medium that drives the medium towards a thermal equilibrium. In addition, for an aqueous solution, the solution having a temperature distribution tends to have a corresponding density distribution. Such a density distribution will also cause the medium to move and to reach equilibrium. The temperature gradient induced motion in the medium is sometimes called thermal convection. Thermal convection may occur within relative large scale within the whole medium, and could be used to facilitate liquid mixing and to act as forces to bring molecules from further distances to certain reaction sites.

Temperature gradient distributions may be established within a MFC-based chamber or apparatus or within an apparatus of the present invention where heating and/or cooling elements may be incorporated into the MFC structures. A heating element may be a simple joule-heating resistor. Such joule-heating resistors could be microfabricated onto the MFC, taking the form of, for example, thin conductive films with defined length and cross-sectional areas. Alternatively, the heating resistors could take the form of microfabricated electrical wires (with defined length and cross-sectional areas) that forms a coil around an insulating body. The heating resistors would have specified resistance values. Take a resistor having a resistance of 10 ohm as an example. Applying 0.2 A

through the coil would result in 0.4 W joule heating-power. When the coil is located in an area < 500 micron^2, this will be an effective way to increase the local temperature and rapidly cause a temperature gradient in the medium. Similarly, a cooling element may be a Peltier element that could reduce local temperatures upon applying electric potentials.

In an exemplary embodiment, the MFC may incorporate an array of individually addressable heating elements. These elements are positioned or structurally arranged in certain order so that when each of or some of or all of elements are activated, temperature gradient distributions can be established to produce desired thermal convection, leading to a velocity field in the medium that is introduced into the apparatus that comprises the MFC. For example, if one heating element is activated or energized, the increase in the temperature in the medium in the neighborhood of this element will result in a local temperature gradient, leading to a thermal convection. In another exemplary embodiment, the MFC may comprise multiple, interconnected heating units so that these units can be turned on or off in a synchronized order. In still another exemplary embodiment, the MFC may comprise only one heating element that can be energized to increase local temperature and induce thermal convection in the medium. Similarly, the MFC may incorporate an array of individually addressable cooling elements, or a single cooling element.

(V.2) Mechanical forces caused by pressure change in the medium

A pressure gradient may be produced in the medium. According to the governing equation for the velocity field in the medium, a pressure gradient, or a change in the pressure within the medium will result in the motion of the medium, leading to a velocity field. The moieties placed in the velocity field will experience physical forces or mechanical forces on the medium.

Various structures may be incorporated into the MFC (multiple-force chip) to produce required pressure gradient or to produce desired pressure change in the medium. One example is a microfabricated pump that is built-in on a MFC so that when the pump is operated, pressure gradient is generated in the medium. This pressure gradient will result in the motion of the medium (e.g., the medium is transported across certain distance in an apparatus), the moieties in the medium will experience mechanical forces and are manipulated by such forces. Another example may be the array of microfabricated tips/capillaries that are incorporated on the MFC. These tips/capillaries may be moved or controlled externally through certain physical mechanisms. For example, these capillaries

or tips are covered with functional materials such as temperature sensitive materials, surface memory alloy, and can thus be further connected with external signal sources or coupled with external physical forces, which will cause the motion of the capillaries or tip. When one or more of these tips/capillaries in the medium are moved, a pressure change and gradient will be produced. Such pressure gradient will lead to the motion of the medium that is in close distance to these tips/capillaries. Moieties in the medium will experience mechanical forces as a velocity field in the medium is generated.

Various internal structures may be used in the apparatus of the present invention. For example, a valve (or microvalve) or pump (or micropump) may be coupled to a reaction chamber that comprise an active biochip that is capable of producing one or more types of physical fields and exerting one or more types of physical forces on the moieties. Such valves or pumps can be operated to produce pressure gradient or pressure change in the medium that is placed in the chamber and to cause a motion in the medium. Thus, these internal structures of the apparatus (that comprises these structural elements plus reaction chambers) may be used to generate a velocity field and to produce mechanical forces on moieties.

(V.3) Mechanical forces caused by body forces exerted on the medium

Velocity field in the medium may be caused by body forces that are applied within the medium. In one example, the substrate surface of a MFC is processed to have appropriate surface charges or appropriate surface charge distribution. The substrate may be used together with other structures (e.g. a cover plate, a gasket or spacer) to form an enclosed chamber with various input/output ports. After a liquid medium is introduced into the chamber, external electrical signal sources may be used to apply a voltage across certain directions in the chamber. The voltage-resultant electric field may interact with the surface charges on the substrate (and/or the surface charges on other surfaces of the chamber) to induce a fluid flow in the liquid medium. The resultant velocity distribution in the positions within the chamber is a velocity field. This velocity field may be used to produce manipulation forces on moieties.

The MFC, the apparatus of the present invention may further include other internal structures that are capable of producing velocity fields and exerting mechanical forces on moieties.

(V.I) Other types of physical forces for manipulating moieties

The multiple-force chip or multiple-force chip-based device, apparatus of the present invention may employ other types of physical forces that are not included in the above list. The inventors envision to use any type of physical forces in MFC structures, as long as they can be externally controlled and can be used with external energy sources to produce physical forces on moieties.

E. Exemplary chips and apparatuses

The present apparatus, or the chip of the present invention (*i.e.*, the multiple-force chip, MFC), may incorporate two types of force-generation elements or structures, such as electrodes for generating electrostatic forces and electromagnetic elements for generating magnetic forces. The combination of two types of forces include, but are not limited to, acoustic force plus electric force (electric force may be electrostatic force or conventional DEP force or traveling-wave DEP force); acoustic force plus magnetic force; acoustic force plus optical force; acoustic force plus mechanical force; electric force plus magnetic force; electric force plus mechanical force; electric force plus optical force; magnetic force plus mechanical force; magnetic force plus optical radiation force; optical-radiation-force plus mechanical force. The total combination number is $C_5^2 = \frac{5*4}{2*1} = 10$ for the case of total five types of forces, such as electric, magnetic, acoustic, mechanical and optical forces. If we divide electric forces into three types, i.e., electrostatic, conventional DEP and traveling wave DEP, then out of the following seven types of forces, i.e., acoustic, magnetic, electrostatic, conventional DEP, traveling-wave DEP, mechanical, optical forces, the combination number of two different forces is $C_7^2 = \frac{7*6}{2*1} = 21$.

The apparatus, or the chip of the present invention (*i.e.*, the multiple-force chip, MFC), may include three types of force-generation elements or structures such as piezoelectric elements or structures for generating acoustic radiation forces, electrode elements for generating traveling-wave DEP forces and electromagnetic elements for generating magnetic forces. The combination of three types of forces include, but are not limited to, acoustic/conventional DEP /magnetic; acoustic/electrostatic/magnetic; acoustic/traveling-wave DEP/mechanical; acoustic/conventional DEP /optical;

acoustic/mechanical/magnetic; acoustic/electrostatic/optical;, acoustic/electrostatic/mechanical, and other combinations. The total combination number $= C_5^3 = \frac{5*4*3}{3*2*1} = 10$ for the case of five types of forces such as electric, magnetic, acoustic, mechanical and optical forces. If we divide electric forces into three types, i.e., electrostatic, conventional DEP and traveling wave DEP, then out of the following seven types of forces, i.e., acoustic, magnetic, electrostatic, conventional DEP, traveling-wave DEP, mechanical, optical forces, the combination number of three different forces is $C_7^3 = \frac{7*6*5}{3*2*1} = 35$.

The apparatus, or the chip of the present invention (*i.e.*, the multiple-force chip, MFC), may include four types of force generation elements or structures such as piezoelectric elements for generating acoustic radiation forces, electrode elements for generating conventional DEP forces, electromagnetic elements for generating magnetic forces and heating elements for generating mechanical forces. Other combination of four types of forces may include acoustic/traveling-wave DEP/magnetic/optical forces and other possible force combinations. The total combination number $= C_5^4 = \frac{5*4*3*2}{4*3*2*1} = 5$ for the case of five types of forces such as electric, magnetic, acoustic, mechanical and optical forces. If we divide electric forces into three types, i.e., electrostatic, conventional DEP and traveling wave DEP, then out of the following seven types of forces, i.e., acoustic, magnetic, electrostatic, conventional DEP, traveling-wave DEP, mechanical, optical forces, the combination number of four different forces is $C_7^4 = \frac{7*6*5*4}{4*3*2*1} = 35$.

The apparatus, or the chip of the present invention (*i.e.*, the multiple-force chip, MFC), may include more than four types of force generation elements or structures. The number of force-type combinations depends on total number N of available force types and the number N_{active} of force types in an apparatus or a MFC given by

$$C_N^{N_{active}} = \frac{N!}{(N - N_{active})! * N_{active}!}.$$

In some cases, different types of forces may be generated/produced/induced through the same force-generation elements or structures. Thus, force-generation elements or structures for different types of forces may be an integral such that, for example, same

electrode elements or structures may be activated to produce electrostatic forces and conventional dielectrophoretic forces acting on molecules and microparticles to be manipulated.

Throughout the present invention, unless specifically indicated otherwise, the force-generation elements or structures are always internal structures of the apparatus, or are always built-in structures located on chip or located on substrates which are part of the chip and the apparatus of the present invention.

Appropriate external signal sources may be applied to these force-generation elements or structures according to required sequence and conditions so that different types of forces may be generated. For example, for a MFC comprising acoustic, conventional DEP and magnetic elements or structures, the sequence of force generation may be as follows: acoustic elements or structures may be activated first for a specified length of time to manipulate all the particles; the magnetic elements or structures may then be activated for a certain length of time to manipulate magnetic particles; the DEP elements or structures may last be activated to manipulate particles having certain dielectric properties.

There may be many different types of embodiment of multiple-force chips, or MFC-based devices, MFC-based apparatuses and systems. In the following we describe some examples of embodiments for multiple-force chips. The descriptions may be readily extended to the MFC-based devices or the apparatus of the present invention.

(E.A) MFC comprising multiple units

One embodiment of multiple-force chip comprises multiple units forming an unit array. Each unit comprises various structural elements that can produce different types of physical forces. In one example, a micro-electromagnetic element or structure, a microelectrode element or structure and a piezoelectric element or structure may be incorporated to form a basic unit. The micro-electromagnetic element or structure may be activated and energized to generate a required magnetic field. The microelectrode element or structure may be energized to produce an appropriate non-uniform electrical field. The piezoelectric element or structure may be energized to generate an acoustic field. In another example, a micro-electromagnetic element or structure, a microelectrode element or structure, a piezoelectric element or structure and a heating element may be incorporated to form a basic unit.

When a MFC comprises multiple units and these units form an unit array, individual units may be essentially identical to each other. Units may be orderly or non-orderly incorporated on a chip. Units may be individually addressable so that each type of elements within each different unit may be selectively addressed so that at any instant time, it is possible to selectively address any type of force element in any one unit. It may also be possible to address all types of the force elements simultaneously within any given unit. Alternatively, some units or all the units may be connected so that the same types of the force elements within these units may be activated or energized simultaneously.

Depending on the applications, different units within the unit array on a MFC may be different so that different units may generate different types of physical forces. For example, a first sub-array of units may comprise electromagnetic elements for producing magnetic fields, a second sub-array of units may comprise micro-electrode elements and acoustic-force elements.

This invention aims at exploiting multiple types of physical forces for manipulating and controlling and processing molecules and particles. It is thus possible that the MFC may comprise any number of units, each unit may comprise any number of different types of force-generation elements. Different units may be identical in their structural elements or may have different structural elements.

For such MFC, different units may be interconnected through certain mechanisms. In one case, the units may be linked through fluidic channels that allow liquid fluid to be transported between these units. The units may also be linked through paths over which the microparticles or molecule-particle complexes, or molecules can be transported. For example, traveling-wave dielectrophoresis electrodes may be used to couple/link different units.

(E.B) MFC comprising overall structures for producing physical forces

Another embodiment of the MFC may comprise overall structures for producing different types of physical forces. For example, the MFC may have one array of electrode elements for generating traveling-wave dielectrophoretic forces; and one array of microelectromagnetic elements for producing magnetic forces; and one piezoelectric element for generating acoustic waves. The difference between such overall structures and the unit-structure-arrays is that when the overall structure is energized, corresponding physical forces are generated nearly-everywhere in the MFC-based chambers or MFC-

based apparatus, whilst the unit-structure-array allows individual units to be activated and the corresponding physical forces are generated only at their corresponding regions within the chamber or the apparatus.

(E.C) MFC comprising overall structures for producing certain types of physical forces and arrays of structural elements for producing other types of forces

Another embodiment of the MFC comprises some overall structure elements for generating certain types of physical forces and some arrays of structural element units for generating other types of physical forces. For example, the MFC may comprise an overall electrode structure that will generate non-uniform electric fields everywhere within the MFC-based chamber or apparatus up on application of electrical signals. Simultaneously, the MFC also comprises an individually addressable micro-electromagnetic unit array that will generate magnetic fields only at the regions close to the units that have been energized/activated.

For the MFC comprising an array of structural element units, different units may be interconnected through certain mechanisms. In one case, the units may be linked through fluidic channels that allow liquid fluid to be transported between these units. The units may also be linked through paths over which the microparticles or molecule-particle complexes, or molecules can be transported. For example, traveling-wave dielectrophoresis electrodes may be used to couple/link different units.

Variation of the MFC structures may further comprise biological/ biochemical elements in different MFC units. Here, the biological/ biochemical elements may include molecules such as protein molecules, nucleotide acids, antibodies and bioparticles such as cells, bacteria, viruses immobilized on the MFC surfaces. These biological/biochemical elements may be uniformly covered on or may be selectively patterned on the MFC surfaces.

F. Exemplary uses

The following illustrates some examples of MFC structures and how they can be utilized for moiety manipulation and for performing biological, biochemical or chemical processing, *e.g.*, separating target cells or molecules from a mixture, transporting target cells or molecules, assaying target cells or molecules, and detecting the assay results, etc.

Figure 1 depicts a schematic drawing for illustrating an apparatus capable of producing four types of physical fields and thus four types of physical forces for manipulating moieties. The apparatus comprises a substrate 100, and four different types of structures that are microfabricated on the substrate 100. The first type of structure comprises microelectrode elements 115, which are capable of generating electric fields E (120) upon application of the externally applied electric signals (not shown in Figure 1). A moiety 126 that is placed in or introduced in the field 120 will experience electric forces 128 (such as electrostatic forces, conventional DEP and/or traveling wave DEP forces). The second type of structure comprises piezoelectric elements 135, which are capable of producing mechanical vibration that can be coupled into the medium that is in direct contact with or in indirect contact with the substrate, resulting an acoustic wave field A (140) in the medium. A moiety 146 that is placed in or introduced in the acoustic field 140 will experience acoustic force 148. The third type of structures comprises an electromagnetic unit 155, which are capable of producing a magnetic field M (160). A moiety 166 that has certain magnetic properties will experience a magnetic force 168 when it is placed into the magnetic field 160. The fourth type of structures comprises a heating element 175, which are capable of generating a temperature gradient in the medium that is in direct contact with or in indirect with the heating element 175, resulting in a velocity field V 180. A moiety 186 will experience a mechanical force 188 when it is placed into the velocity field 180.

The four types of structures in Figure 1 are fabricated on the substrate 100. In an alternative approach, the structures may take different forms and may be attached onto or bound to the substrate 100.

Figure 1 shows an example of multiple-force chips that are capable of generating four types of forces. Figure 2 depicts a multiple-force chip 200 that comprises an array of individual units 210. Each unit 210 consists of several types of force-generation structures or elements including an electrical element/structure EL (220) for producing an electric field, a magnetic element/structure MAG (260) for producing a magnetic field, an acoustic element/structure ACS (240) for producing an acoustic field. Each unit 210 further comprises a biological element BIO (270). The biological elements may be molecules or biomolecules that are attached or immobilized on the chip surface. The biological elements may be used for certain biological/biochemical reactions on the chip. For example, the moieties to be manipulated are single stranded DNA target molecules, and the biological

elements 270 may be single stranded probe DNA molecules immobilized on the chip surface. When the target DNA molecules are manipulated onto the chip surface through various types of forces such as electric, magnetic or acoustic forces with the elements shown in Figure 2, the target DNA molecules may hybridize with or bind to the probe DNA molecules under appropriate conditions if they are complementary to each other. Such hybridization reactions can then be detected by appropriate means.

In Figure 2, it is shown that all the units are identical within the chip. However, this is not necessary. In other examples, different units on the multiple-force chip may comprise different types of structures/elements. One unit may comprise a magnetic element/structure plus an acoustic element; another unit may comprise an electric element. Figure 2 also shows that the four elements within a unit occupy separate positions. However, this is not necessary. In other examples, different structure elements within one unit may overlap and occupy same positions on the chip. For example, within a unit, the electric elements may be over the acoustic elements.

Figures 3A, 3B and 3C show three different embodiments of two-force chips of the present invention, capable of producing acoustic force and conventional dielectrophoretic (DEP) forces. In Figure 3A, a substrate 300 made of piezoelectric materials (such as piezoelectric ceramics PZT) is used. Any types of piezoelectric materials may be used provided that they can generate sufficient mechanical vibrations upon the application of electric signals. A number of commercially available piezoelectric discs or substrates may be used. The appropriate thickness of the piezoelectric substrate should be used in order to generate acoustic wave fields of required frequencies. Examples of the substrate materials can be found in a number of articles, including Yasuda K. et al, *J. Acoust. Soc. Am.* Vol. 102 (1), pages 642-645, July, 1997; Yasuda K. and Kamakura T. *Appl. Phys. Lett,* Vol. 71(13), pages1771-1773, Sep. 1997; Pui et al, *Biotechnol. Prog.*, 11:146-152 (1995); Yasuda et al, *J. Acoust. Soc. Am.*, Vol. 99(4), pages: 1965-1970 (1996); and Yasuda et al., *Jpn. J. Appl. Phys.*, Vol. 35(1), pages: 3295-3299 (1996). As shown in Figure 3A, one major surface 320 of the substrate 300 is covered with an electric conductive layer (e.g., thin metal film) that serves as one electrode for connecting to the external signals to energize the piezoelectric substrate.

In Figure 3A, an interdigitated electrode array 340 is fabricated on the other major surface of the substrate 300. The electrode array comprises two sets of line-type electrode elements. The electrode elements within each set are connected together. So there are two

electrical connection pads 342 and 346 that are connected to the two sets of the electrodes. The electrode array 340 may be fabricated on the substrate 300 using photolithography with a single photomask, or may be fabricated using other microfabrication methods or micromachining protocols, as known to those skilled in the art of microlithography and microfabrication (*See, for example,* Rai-Choudhury P. (Editor), Handbook of Microlithography, Micromachining and Microfabrication, Volume 2: Micromachining and microfabrication. SPIE Optical Engineering Press, Bellingham, Washington, USA (1997)). The protocols in the microfabrication may include many basic steps, for example, photolithographic mask generation, metal deposition, insulator deposition, photoresist deposition, photoresist patterning with masks and developers, metal or insulator layer patterning. Electrodes may be made of metal materials such as aluminum, gold, silver, tin, copper, platinum, palladium and carbon, semiconductor materials such as phosphorous-doped silicon, and any other materials as along as they have high electrical conductivities. Those who are skilled in microfabrication and micromachining fabrication may readily choose and determine the fabrication protocols and materials that should be used for fabrication of such electrode structures. The electrode structures shown in Figure 3A are interdigitated electrodes, however, many other electrode structures could be used. For example, the electrodes may be interdigitated castellated electrodes, spiral electrodes, polynomial electrodes and others. In photolithographic fabrication of electrodes with different geometries, many fabrication steps remain to be the same, with the difference being the use of masks with appropriate geometry that corresponds to the required electrode array.

The piezoelectric substrate 300 in Figure 3A can be energized by external signal source 360 through the connections on the surface 320 of the substrate 300 and the connection pad 346 on the substrate 300. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied to the substrate, mechanical vibrations are generated from the substrate 300 and can be coupled into the medium (not shown in Figure 3A) that may be in direct contact or in indirect contact with the substrate 300, producing an acoustic wave field in the medium. The moiety in the medium will experience acoustic forces from the acoustic field. Note that for such a two-force chip, only certain regions of the piezoelectric substrate are caused to vibrate. These regions correspond to the electrodes that are connected to the connection pad 346. The electrode array 340 on the substrate 300 in Figure 3A may be energized by

external signal source 380 through connection pads 342 and 346 to produce a non-uniform electric field in a medium that is in direct or indirect contact with the electrode array. The signals supplied by the signal source 380 may have frequencies between less than 1 Hz and above 1 GHz. The electric field in the medium may exert electric forces (e.g. conventional DEP forces) on the moieties that are placed in the field.

The surface of substrate 300 that has been processed to contain the electrode array 340 may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 300, the medium is in indirect contact with the substrate 300. The indirect contact between the medium and the substrate 300 is through the layer of functional materials (and through the electrode array 340 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 300. Examples of a functional layer include, but not limited to, a molecular monolayer, a membrane, a gel, a porous or non-porous material layer. The functional layer may be an additional layer adhered to the substrate surface. Alternatively, the functional layer may be formed by direct chemical-modification of the substrate surfaces. Ideally, the functional layer should have minimal or no non-specific bindings to molecules other than molecules that are required for immobilization, should allow efficient binding or attachment of required molecules. The specific molecules incorporated in the functional layer for binding required molecules to the substrate surfaces are referred to as the functional groups. Examples of the functional groups include, but not limited to aldehydes, carbodiimides, succinimydyl esters, antibodies, receptors, and lectins. The functional groups also include chemical groups or molecular sites that are formed through chemical modification on the chip surface molecules. The functional layers may be selected from the group consisting of a hydrophilic molecular monolayer, a hydrophilic molecular monolayer with functional groups, a hydrophobic molecular monolayer, a hydrophobic molecular monolayer with functional groups, a hydrophilic membrane, a hydrophilic membrane with functional groups, a hydrophobic membrane, a hydrophobic membrane with functional groups, a hydrophilic gel, a hydrophilic gel with functional groups, a hydrophobic gel, a hydrophobic gel with functional groups, a porous material, a porous material with functional groups, a non-porous material and a non-porous material with functional groups.

In Figure 3B, the two-force chip comprises a substrate 300 and a substrate 355 bound together or attached together. The substrate 300 is made of piezoelectric materials

(such as piezoelectric ceramics PZT). Any types of piezoelectric materials that are described in the context of Figure 3A may be used. As shown in Figure 3B, both major surfaces 320 and 350 of the substrate 300 are covered with an electric conductive layer (e.g., thin metal film) that serves as electrodes to energize the piezoelectric substrate. The two conductive surfaces may be connected to an external signal source 360. An array 370 of circle electrode elements is fabricated on a second substrate 355. The electrode array comprises two sets of interconnected circle-type electrode elements. The electrode elements within each set are connected together. So there are two electrical connection pads 372 and 376 that are connected to the two sets of the electrodes. The substrate 355 with fabricated electrode array 370 on its surface is attached to, or bound to, or linked to the piezoelectric substrate 300. Careful binding between the piezoelectric substrate 300 and the substrate 355 is necessary so that acoustic wave field may be coupled from the piezoelectric substrate 300 through the substrate 355 to the medium (not shown in Figure 3B) that is in direct or indirect contact with the substrate 355. Binding between the two substrates preferably is complete so that no air gap exists between the two substrates. One method to bind substrates is by gluing them together.

The piezoelectric substrate 300 in Figure 3B can be energized by external signal source 360 through the connections on the surfaces 320 and 350 of the substrate 300. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied to the substrate, mechanical vibrations are generated from the substrate 300 and can be coupled through the substrate 355 into the medium (not shown in Figure 3B), producing an acoustic wave field in the medium. The medium may be in direct or indirect contact with the medium. The moiety in the medium will experience acoustic forces due to the acoustic field. The electrode array 370 on the substrate 355 in Figure 3B may be energized by external signal source 380 to produce a non-uniform electric field in a medium that is in direct or indirect contact with the electrode array. The signals supplied from the signal source 380 may have frequencies between less than 1 Hz and above 1 GHz. The electric field in the medium may exert electric forces (e.g. conventional DEP forces) on the moieties that are placed in the field.

The surface of the substrate 355 in Figure 3B that has been processed to contain an electrode array 370 may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 355 (and the substrate 300), the medium is in indirect contact with the substrate

355. The indirect contact between the medium and the substrate 355 is through the layer of functional materials (and through the electrode array 370 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 355. Some examples of the functional layer have been provided in the context of describing Figure 3A.

In Figure 3C, the two-force chip comprises a substrate 390 and a substrate 355 bound together or attached together. The substrate 390 can be made of any solid material such as silicon, glass, plastics etc, provided that the substrate 390 can be microporocessed or micromachined to form channels, wells on the substrate. The substrate 390 is, preferably, not made of piezoelectric materials. An array of piezoelectric elements 395 (i.e. an array of acoustic wave sources) are fabricated into the substrate 390. The fabrication process may be as follows. The substrate 390 is etched to certain depth at predetermined positions to form an array of circular wells on the substrate. Each well has a predefined dimension in terms of the diameter of the circle (e.g., from 50 micron to several mm) and the depth of the wells (e.g., between 5 micron and 10 mm). This etching step may involve the use of photolithography or other micromaching processes. The etched wells are connected together through the channels that have the same depth as the wells. A thin film (e.g. between 50 nm and several micron) of conductive materials (e.g. gold, titanium) may then be deposited on the substrate, covering the etched wells and the etched channels. Thus, the thin conductive layers on the bottom of the etched wells and channels are continuous and are electrically connected. Following this step, a thin film of piezoelectric materials is deposited on the substrate, and the thickness of the thin film is preferably smaller than the depth of the etched wells on the substrates. Certain processing step is used to remove the metal film and piezoelectric film that are on the regions of the substrate except those in the etched wells and channels. A thin conductive layer is deposited again on the substrate, covering all the wells and channels. This conductive layer is so thick that the conductive layer is electrically-connected everywhere through the whole surface of the substrate. Thus, we have two electrically conductive layers, one being on the bottom of the etched wells and channels, one being on the surface of the substrate. Between these two conductive layers, there is an array of circular-discs that are made of piezoelectric materials. Thus on the substrate 390 in Figure 3C, we have formed an array of piezoelectric elements 395 that are connected together in parallel through the electrodes sandwiching the piezoelectric discs. The electrically conductive layers can be connected to

an external signal source 360 through the connection pads 392 and 398. The above process for making the array of piezoelectric elements 395 in the non-piezoelectric substrate is for illustration only. Any microfabrication methods or any micromachining protocols that are suitable for producing thin film piezoelectric materials in a substrate may be used. Those who are skilled in the fabrication of an acoustic array may readily choose appropriate methods, processes, protocols and materials for fabricating such an array of acoustic wave sources (or an array of piezoelectric elements) on a substrate. Other structures of the piezoelectric elements may be used as well. For example, the etched wells may be of any other shape, such as square, rectangular, diamond, etc, and may be of any dimensions that are suitable for producing required acoustic waves. The fabricated array of piezoelectric elements does not have to be buried into the substrate. Indeed they can be formed on or above the surfaces of the substrate. All the fabricated piezoelectric elements do not have to connect together. For example, some of the elements may be connected together. In another examples, the individual elements may be selectively addressable.

In Figure 3C, an interdigitated electrode array 340 is fabricated on a second substrate 355. The electrode array has similar structures to the interdigitated electrode array shown in Figure 3A, comprises two sets of line-type electrode elements. The electrode elements within each set are connected together. So there are two electrical connection pads 342 and 346 that are connected to the two sets of the electrodes. The substrate 355 with fabricated electrode array 340 on its surface are attached to, or bound to, or linked to the substrate 390. Careful binding between the substrate 390 and the substrate 355 is necessary so that acoustic wave field may be coupled from the substrate 390 through the substrate 355 to the medium (not shown in Figure 3B) that is in direct or indirect contact with the substrate 355. Binding between the two substrates preferably is complete so that no air gap exists between the two substrates. One method to bind the substrates 390 and 355 together is by gluing them together. Another method may be through certain bonding approaches such as anodic boding.

The piezoelectric elements 395 on the substrate 390 in Figure 3C can be energized by external signal source 360 through the connection pads 392 and 398. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied to the piezoelectric elements 395, mechanical vibrations are generated from these elements and can be coupled through the substrate 355 into the medium (not shown in Figure 3C) that may be in direct contact or in indirect contact with

the substrate 355, producing an acoustic wave field in the medium. In such a way, each piezoelectric element 395 serves as an acoustic wave source. The moiety in the medium will experience acoustic forces from the acoustic field. The electrode array 340 on the substrate 355 in Figure 3B may be energized by the external signal source 380 to produce an electric field in the medium. The electric field in the medium may exert electric forces (e.g. conventional DEP forces) on the moieties that are placed in the field.

The surface of the substrate 355 that has been processed to contain the electrode array 340 in Figure 3C may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 355 and substrate 390, the medium is in indirect contact with the substrate 355. The indirect contact between the medium and the substrate 355 is through the layer of functional materials (and through the electrode array 340 in certain regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 355. Some examples of the functional layer have been provided in the context of describing Figure 3A.

To use the multiple-force chips of the present invention (e.g. those illustrated in Figures 1, 2 3A-3C) for moiety manipulation with various manipulation methods, a fluidic chamber or an apparatus for manipulating moieties may be constructed. Figure 4 shows an example of such fluidic chambers. Here, the chamber comprises a multiple-force chip 500 on the bottom, a spacer 520 that is cut in the middle to define the chamber thickness, a top plate 550 that has input fluidic input port 560 and output port 580 incorporated on the plate 550. These three parts are bond together to form a fluidic chamber. For illustration, these three parts are not drawn together. The multiple-force chip has various built-in structures incorporated on it surface or its substrate. For clarity, no detailed structures are shown in Figure 4, and for demonstration purpose, the schematic representation of the multiple-force chip in Figure 2 is used here. It shows that the multiple-force chip 500 in Figure 4 comprises an array of units and each unit is capable of producing electric field, magnetic field and acoustic field. Furthermore, each unit further comprises biological elements.

The fluidic chamber shown in Figure 4 is for illustration purpose only. Fluidic chambers or manipulation apparatuses can be constructed according other arrangements, rather than those shown in Figure 4. For example, the spacer 520 may be a part of the top plate 550. Thus, the top plate 550 is processed to have a defined channel formed. One example is that the top plate is a glass substrate. One surface of the glass substrate may be

etched in a region to form a channel with defined thickness. The basic requirement of the fluidic chamber or manipulation apparatus are: a substrate that can hold or support moieties to be manipulated and internal structures that are capable of producing multiple types of forces on moieties. The fluidic chamber may comprise a housing that can be combined with the substrate and be used to contain the moieties to be manipulated. The fluidic chamber may be an open chamber or an enclosed chamber. The chamber may have top, bottom and side walls. One example of such chambers is shown in Figure 4. The chamber may have a spacer or gasket. The chamber may comprise a multiple-force chip at the chamber bottom. The chamber may comprise a chip capable of producing at least one type of physical fields/forces at the chamber bottom and another chip capable of producing at least one type of physical fields/forces at the chamber top. Preferably, for a closed chamber, the chamber comprises at least one inlet port and one outlet port. The inlet port is used for introducing sample that contains moieties to be manipulated into the chamber. The outlet port is used for removing the sample from the chamber. For an open chamber, the inlet port and the outlet port may be an integral. The chamber may be an open chamber with the bottom walls and side walls, and becomes a closed chamber after the sample is introduced and the chamber is covered with a top plate. The close chamber with the sample loaded inside is then fully enclosed and can be used for experiments.

Many types of fluidic chambers, flow cells, and fluidic apparatuses that have incorporated chips or biochips have been described in literature. The structural arrangements of those devices described in the literature can be adopted to a fluidic chamber, or manipulation apparatus that comprises a multiple-force chip of the present invention. Some examples of the articles describing fluidic chambers are as follows: "Dielectrophoretic manipulation of particles by Wang *et al*, in *IEEE Transaction on Industry Applications*, Vol. 33, No. 3, May/June, 1997, pages 660-669"; "Separation of human breast cancer cells from blood by differential dielectric affinity by Becker *et al*, in *Proc. Natl. Acad. Sci.*, Vol., 92, January 1995, pages 860-864"; "Selective dielectrophoretic confinement of bioparticles in potential energy wells by Wang *et al.* in J. Phys. D: Appl. Phys., Volume 26, pages 1278-1285"; "Ultrasonic manipulation of particles and cells" by Coakley *et al. Bioseparation.* 1994, Vol. 4, pages: 73-83", "Particle column formation in a stationary ultrasonic field" by Whitworth *et al., J. Accost. Soc. Am.* 1992, Vol. 91, pages: 79-85", "Electrokinetic behavior of colloidal particles in traveling electric fields: studies using yeast cells by Huang *et al*, in *J. Phys. D: Appl. Phys.*, Vol. 26, pages 1528-

1535", "Positioning and manipulation of cells and microparticles using miniaturized electric field traps and traveling waves. By Fuhr *et al.*, in *Sensors and Materials*. Vol. 7: pages 131-146, 1995", "Dielectrophoretic manipulation of cells using spiral electrodes by Wang, X-B. *et al.*, in *Biophys. J.* Volume 72, pages 1887-1899, 1997"; "Preparation and hybridization analysis of DNA/RNA from *E. coli* on microfabricated bioelectric chip by Cheng, J. *et al.,* in *Nature Biotech.*, Volume, 70, pages: 2321-2326, 1998"; "Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow-fractionation by Yang, J. *et al.* in *Anal. Chem.* Vol. 71: pages, 911-918, 1999".

Figures 5A-5C illustrates examples of two-force chips of the present invention, capable of producing acoustic forces and magnetic forces for manipulating moieties. Figure 5A shows that an array of microelectromagnetic units or electromagnetic units for producing magnetic forces is fabricated on a substrate 300. The substrate 300 is made of piezoelectric materials (such as piezoelectric ceramics PZT) and is capable of generating acoustic fields. Any types of piezoelectric materials that are described in the context of Figure 3A may be used. Both major surfaces of the substrate 300 are covered with an electric conductive layer (e.g., thin metal film) that serves as electrodes to energize the piezoelectric substrate. In Figure 5A, only the electric conductive layer 320 on the bottom surface is shown and the conductive layer on the top surface is not shown for the clarity. Nevertheless, a connection pad 435 was shows on the top surface of the substrate 300. Thus, electrical signals from signal source 360 may be connected to the electrodes 435 and 320 to energize the piezoelectric substrate 300. An insulating, dielectric layer (not shown in Figure 5A) is deposited over the conductive layer on the top surface of the substrate 300. This dielectric layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to about 200 microns. Preferably, the dielectric layer is between 3 micron and 50 micron. An array of microelectromagnetic units 415 is then fabricated on the insulating dielectric layer. For the fabrication of electromagnetic units, the insulating dielectric layer should be processed properly, for example, having a smooth surface or being polished properly. The microelectromagnetic units may take the form of any shape and any structural arrangement, as long as the unit is capable of producing a magnetic field upon application of electric current. Each unit may have various geometries, for example, the geometries of electromagnetic units disclosed in co-pending US patent application serial No: 09/399,299, filed on September 16, 1999. Other examples of electromagnetic units

that can be incorporated in the chip shown in Figure 5A include, but not limited to, the structures described in the following articles or publications: Ahn, C., *et al.*, *J. Microelectromechanical Systems*. Volume 5: 151-158 (1996); Ahn, C., *et al.*, *IEEE Trans. Magnetics*. Volume 30, pages, 73-79 (1994); Liakopoulos *et al.*, in *Transducers 97,* pages 485-488, presented in 1997 International Conference on Silid-State Sensors and Actuators, Chicago, June 16-19, 1997; US patent No. 5,883,760 by Naoshi *et al.* Various microfabrication protocols and methods such as those described in the articles and publications cited immediately above may be employed to fabricate these electromagnetic units. Many examples and principles of microlithography and microfabrication are shown in "Handbook of Microlithography, Micromachining and Microfabrication, by Rai-Choudhury P. (Editor), Volume 2: Micromachining and microfabrication. SPIE Optical Engineering Press, Bellingham, Washington, USA (1997)". Based on the specific geometry and other requirements (e.g. required magnetic field strength) of the electromagnetic units, those who are skilled in the microfabrication on substrates and in the magnetic thin filming processing may readily choose appropriate materials and methods for fabricating electromagnetic units.

Electromagnetic units 415 in the array shown in Figure 5A may be individually addressable. Alternatively, some of the units, or all the units may be connected together in parallel. Various means to selectively addressing individual units in the array, such as those described in the co-pending US patent application serial No: 09/399,299, filed on September 16, 1999, may be employed. Electric connection pads 440 that are fabricated together with the electromagnetic units 415 are shown in Figure 5A. For clarity, we only show the electrical connection between some connection pads and some electromagnetic units 415, although all the units have corresponding connection pads if they are individually addressable. Through these connection pads 440, electric current (DC or AC) from externally applied electric current sources 450 can be connected to the electromagnetic units 415 so that the units 415 are energized to produce magnetic fields.

The piezoelectric substrate 300 in Figure 5A can be energized by external signal source 360 through the connections on pad 435 and conductive surface 320. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied, mechanical vibrations are generated from the piezoelectric substrate and can be coupled into the medium (not shown in Figure 5A) that may be in direct contact or in indirect contact with the substrate 300, producing an acoustic

wave field in the medium. The moiety in the medium will experience acoustic forces from the acoustic field. The array of electromagnetic units 415 in Figure 5A may be energized by external current source 450 to produce magnetic fields in the medium. The magnetic field in the medium may exert magnetic forces on the moieties that have certain magnetic properties.

The surface of the substrate 300 that has been processed to contain an array of electromagnetic units as shown in Figure 5A may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 300, the medium is in indirect contact with the substrate 300. The indirect contact between the medium and the substrate 300 is through the layer of functional materials (and through the electromagnetic units in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 300. Some examples of the functional layer have been provided in the context of describing Figure 3A.

Figure 5B shows that an array of acoustic wave sources for producing acoustic waves is fabricated on a solid piezoelectric substrate 300. A microelectromagnetic unit array is fabricated on a second substrate 355. The second substrate is then bond to the first acoustic substrate, forming a multiple-force chip capable of producing acoustic forces and magnetic forces. The array of acoustic wave sources is formed by fabricating an electrode array 460 on one surface (the top surface in Figure 5B) of the piezoelectric substrate 300. The electrode array 460 in Figure 5B is an array of circle electrodes that are all connected together through electrode lines 468. The electrodes of other geometries may also be used, depending on the requirement for the acoustic wave sources. Although the electrodes in Figure 5A are shown connected together, electrodes in the array may be individually addressable, or some electrodes in the array may be connected together. The other surface 320 (the bottom surface in Figure 5B) is covered with a thin film of conductive materials. When AC electrical signals from the external signal source 360 are applied to the electrode surface 320 and electrode array 460 through connection pads 462, the regions in the substrate 300, which are sandwiched between the surface 320 and the electrode array 460, will be subjected to the applied AC electric field and produce mechanical vibrations. The methods for fabricating the electrode array 460 on the piezoelectric substrate are similar to those for fabricating the interdigitated electrode array shown in Figure 3A.

An array of electromagnetic units 415 is fabricated on a second substrate 355, as shown in Figure 5B. The geometry and structure of the electromagnetic unit 415, the layout of the array, and the methods for fabricating such electromagnetic unit array are similar to those described for electromagnetic unit array shown in Figure 5A. The substrate 355 that has been processed to contain the array of electromagnetic units 415 is bound to the piezoelectric substrate 300. Such binding between the two substrates 300 and 355 should be carefully done to ensure that acoustic wave can be coupled from the substrate 300 through the substrate 355 into the medium (not shown in Figure 5B). The methods for binding these two substrates together are similar to those described in the context of Figure 3B for binding the acoustic substrate and the dielectrophoretic-electrode containing substrate.

The piezoelectric substrate 300 in Figure 5B can be energized by external signal source 360 through the connections on pad 462 and conductive surface 320. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied, mechanical vibrations are generated from the array of piezoelectric transducer sources and can be coupled into the medium (not shown in Figure 5B) through the substrate 355, producing an acoustic wave field in the medium. The medium may be in direct contact or in indirect contact with the substrate 355. The moiety in the medium will experience acoustic forces due to the acoustic field. The array of electromagnetic units 415 in Figure 5B may be energized by external current source 450 to produce a magnetic field in the medium. The magnetic field in the medium may exert magnetic forces on the moieties that have certain magnetic properties.

The surface of the substrate 355 that has been processed to contain an array of electromagnetic units as shown in Figure 5B may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 355 (and the substrate 300), the medium is in indirect contact with the substrate 355. The indirect contact between the medium and the substrate 355 is through the layer of functional materials (and through electromagnetic units in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 355. Some examples of the functional layer have been provided in the context of describing Figure 3A.

Figure 5C shows that an array of microelectromagnetic units 415 is co-fabricated with an array of piezoelectric disc elements 395 on a solid substrate 390. The method for

fabricating the piezoelectric elements 395 is similar to that described for fabricating the piezoelectric elements in the context of Figure 3C. The method for fabricating electromagnetic units is similar to that used for fabricating the electromagnetic units in Figure 5A and 5B. These fabrication methods may be combined together. Those who are skilled in microfabrication and in magnetic thin film processing may readily determine and adopt appropriate fabrication protocols for making these piezoelectric elements 395 and electromagnetic elements 415 together on one substrate. The piezoelectric elements 395 are shown in Figure 5C having disc geometries, but they may be any other shape as determined by the requirement for the acoustic field. Similarly, the geometry and structure of the electromagnetic units 415 may be of various forms. Non-limiting examples of these variations have been provided in the context of Figure 5A.

The piezoelectric elements 395 in Figure 5C can be energized by external signal source 360 through the connections on pads 392 and 398. When AC signals of appropriate waveforms (typically sinusoidal signal, at appropriate frequencies) from the signal source 360 are applied, mechanical vibrations are generated from the piezoelectric elements 395 and can be coupled into the medium (not shown in Figure 5B) that may be in direct contact or in indirect contact with the substrate 390, producing an acoustic wave field in the medium. The moiety in the medium will experience acoustic forces from the acoustic field. The array of electromagnetic units 415 in Figure 5C may be energized by external current source 450 to produce a magnetic field in the medium. The magnetic field in the medium may exert magnetic forces on the moieties that have certain magnetic properties.

The surface of the substrate 390 that has been processed to contain an array of electromagnetic units 415 and an array of piezoelectric elements 395 as shown in Figure 5C may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 390, the medium is in indirect contact with the substrate 390. The indirect contact between the medium and the substrate 390 is through the layer of functional materials (and through electromagnetic units or piezoelectric elements in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the substrate 390. Some examples of the functional layer have been provided in the context of describing Figure 3A.

Figures 6A-6D show schematic drawing of examples of two-force chips of the present invention, capable of producing dielectrophoretic (DEP) forces (conventional DEP and/or traveling-wave DEP forces) and magnetic forces. Figure 6A shows that an array of

microelectromagnetic units 415 is fabricated on a substrate 355. The geometry and structure of the electromagnetic unit 415, the layout of the array, and the methods for fabricating such electromagnetic unit array are similar to those described for electromagnetic unit array shown in Figure 5A. After the array of electromagnetic units 415 is fabricated, the substrate 355 is further covered with a thin dielectric layer 460. This layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to about 200 microns. Preferably, the dielectric layer is between 1 micron and 10 micron. Note for clarity, the dielectric layer 460 is shown separated from the substrate 355. The surface of dielectric layer 460 is further processed or polished for smoothness so that an interdigitated electrode array 340 can be fabricated on the dielectric layer 460. The structure of the electrodes 340, the layout of the array, and the methods for fabricating this interdigitated electrode array are similar to those described for the electrode array shown in Figure 3A and 3C. Note that after the fabrication of electrode array 340, it is necessary to ensure that the connection pads 440 for the electromagnetic units 415 on the substrate 355 are still open to external connection. This can be achieved by either protecting these pads 440 during the fabrication process, alternatively, can be achieved through proper photolithography procedures.

The electromagnetic elements 415 in Figure 6A can be energized by electric current (DC or AC) from external signal source 450 (i.e., current source) through the connections on pad 440. Magnetic fields will be generated in the neighborhood of electromagnetic units. The magnetic field will penetrate through the dielectric layer 460 into the medium (not shown in Figure 6A) that may be in direct contact or in indirect contact with the dielectric layer 460. The magnetic field in the medium may exert magnetic forces on the moieties that have certain magnetic properties. The electrodes 340 in Figure 6A may be energized by electric signals (typically AC, from below 1 Hz to above 1 GHz) signal source 380 to produce a non-uniform electric field in the medium. The non-uniform electric field will exert conventional DEP forces on the moieties in the medium as long as the moieties have different dielectric properties from those of the medium.

The surface of the dielectric layer 460 that has been processed to support the electrode array 340 as shown in Figure 6A may be further covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 355 and the dielectric layer 460, the medium is in indirect contact

with the dielectric layer 460. The indirect contact between the medium and the dielectric layer 460 is through the layer of functional materials (and through the electrode array 340 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the dielectric layer 460. Some examples of the functional layer have been provided in the context of describing Figure 3A.

The multiple-force chips shown in Figure 6B, Figure 6C and Figure 6D are similar to those shown in Figure 6A, with the differences being in the structures of electrodes on the dielectric layer 460.

In Figure 6B, a four phase, linear, traveling-wave dielectrophoresis electrode array 480 is fabricated on the layer 460. The layout and structural arrangement of the array 480 is similar to those described in literature, for example in "Positioning and manipulation of cells and microparticles using miniaturized electric field traps and travelling waves", G. Fuhr et al., *Sensors and Materials,* vol. 7, pages 131-146, 1995; or in "Large-area traveling-wave dielectrophoretic particle separator", Morgan et al., *J. Micromech. Microeng.* Volume 7, pages 65-70, 1997". Although not shown in Figure 6B, the electrode array 480 has multiple conductive layers. The photolithography process for fabricating such electrodes, if used, involves multiple photomasks. Again, those who are skilled in microfabrication can readily choose and determine appropriate protocols and procedures to fabricate such electrode array 480. The electrode array 480 can be energized by four-phase signals (0, 90, 180 and 270 degrees for a sinusoidal or near sinusoidal waveform) supplied from a signal generator 490 through connection pads 486 to generate non-uniform, traveling wave electric fields. Such a field can exert both conventional DEP forces and traveling-wave DEP forces on moieties that are introduced in the field.

In Figure 6C, an array of diamond-shaped electrodes 610 is fabricated on the dielectric layer 460. All the electrode elements are divided (and connected up) into two sets, with electrodes in each set connected together, as shown in Figure 6C. Such electrodes can be fabricated using the methods for fabricating the interdigitated electrodes shown in Figure 3A (e.g. using photolithography with appropriate photomasks). The diamond-electrode array 610 can be energized by electric signals (typically AC from below 1 Hz to above 1 GHz) supplied from a signal generator 380 through connection pads 612 and 618 to generate a non-uniform electric field. Such a field can exert conventional DEP forces on moieties that are introduced in the field.

In Figure 6D, a spiral electrode array 630 is fabricated on the dielectric layer 460. There are four, parallel, linear spiral electrode elements in the spiral array 630. Such electrodes can be fabricated using the methods for fabricating the interdigitated electrodes shown in Figure 3A (e.g. using photolithography with appropriate photomasks). The spiral electrode array 630 can be energized by four-phase signals (0, 90, 180 and 270 degrees for a sinusoidal or near sinusoidal waveform) supplied from a signal generator 490 through connection pads 636 to generate non-uniform, traveling wave electric fields. Such a field can exert both conventional DEP forces and traveling-wave DEP forces on moieties that are introduced in the field. The geometry of a spiral electrode array, the principle and the operation using a spiral electrode array for cell manipulation have been described previously in the article "Dielectrophoretic manipulation of cells with spiral electrodes, X-B. Wang et al., *Biophysical J.*, vol. 72, pages 1887-1899, 1997".

The multiple-force chips shown in Figures 6A, 6B, 6C and 6D are all capable of producing magnetic fields (thus magnetic forces on moieties with certain magnetic properties) and producing electric fields of non-uniform magnitude distribution (thus conventional DEP forces). The chips in Figure 6B and Figure 6D are also capable of producing traveling wave electric fields (thus traveling-wave DEP forces).

Figures 7A and 7B show schematic drawing of examples of two-force chips of the present invention, capable of producing electrostatic (or electrophoretic) forces and magnetic forces. Figure 7A shows that an array of microelectromagnetic units 415 is fabricated on a substrate 355. The geometry and structure of the electromagnetic units 415, the layout of the array, and the methods for fabricating such electromagnetic unit array are similar to those described for electromagnetic unit array shown in Figure 5A. After the array of electromagnetic units 415 is fabricated, the substrate 355 is further covered with a thin insulating, dielectric layer 460. This layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to about 200 microns. Preferably, the dielectric layer is between 1 micron and 10 micron. Note for clarity, the dielectric layer 460 is shown separated from the substrate 355. The surface of dielectric layer 460 is further processed or polished for smoothness so that an individually addressable electrode array 660 can be fabricated on the dielectric layer 460. Here each electrode element has a rectangular shape (with dimensions from several micron, e.g. 1 micron by 5 micron to several mm, e.g. 1 mm by 3 mm). Each electrode element can be addressed externally with electrical signals

through connection pads 662 shown in Figure 7A. For clarity, in Figure 7A, we only show the connections between some electrode elements and connection pads. The methods for fabricating this electrode array are similar to those described for the electrode array shown in Figure 3A and 3C. Note that after the fabrication of electrode array 340, it is necessary to ensure that the connection pads 440 for the electromagnetic units on the substrate 355 are still open to external connection. This can be achieved by either protecting these pads 440 during the fabrication process, alternatively, can be achieved through proper photolithography procedures.

The electromagnetic elements 415 in Figure 7A can be energized by electric current (DC or AC) from external signal source 450 through the connections on pad 440. Magnetic fields will be generated in the neighborhood of electromagnetic units. The magnetic field will penetrate through the dielectric layer 460 into the medium (not shown in Figure 7A) that may be in direct contact or in indirect contact with the dielectric layer 460. The magnetic field in the medium may exert magnetic forces on the moieties that have certain magnetic properties. The electrode array 660 in Figure 7A may be energized by electric signals (typically DC or low frequency AC, from below 1 Hz to several kHz) from signal source 680 through connection pads 662 to produce a DC (or low frequency AC) electric field in the medium. The electric field will exert electrostatic forces on the moieties in the medium as long as the moieties have net electric charges.

The surface of the dielectric layer 460 that has been processed to support the electrode array 660 as shown in Figure 7A may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 355 and the dielectric layer 460, the medium is in indirect contact with the dielectric layer 460. The indirect contact between the medium and the dielectric layer 460 is through the layer of functional materials (and through the electrodes 660 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the dielectric layer 460. Some examples of the functional layer have been provided in the context of describing Figure 3A.

The multiple-force chips shown in Figure 7B are similar to those shown in Figure 7A, with the difference being in the structures of electrodes on the dielectric layer 460. In Figure 7B, a four phase, linear, traveling-wave electrophoresis electrode array 480 is fabricated on the layer 460. The geometry and structure of the electrode array 480, the layout of the array, and the methods for fabricating such electrode unit array are similar to

those described for electrode array shown in Figure 6B. The electrode array 480 in Figure 7B may be energized through connection pads 486 by phase-shifted electric signals (typically pulsed DC) from a signal source 700 to produce a traveling wave DC electric field in the medium. The electric field will exert traveling-wave electrophoretic (or traveling-wave electrostatic) forces on the moieties in the medium as long as the moieties have net electric charges. The operation principle of such traveling wave electrophoretic movement of charged moieties was described in the co-pending U.S. Application "METHODS FOR MANIPULATING MOIETIES IN MICROFLUIDIC SYSTEMS" (US patent application no. 09/636,104) by Wang et al, filed on August 10, 2000.

Figures 8 shows schematic drawing of examples of two-force chips of the present invention, capable of producing electrophoretic forces and thermal convection-induced mechanical forces. Figure 8 shows that an array of individually addressable, electrically-heating elements 750 is fabricated on a solid substrate 720. The substrate 720 may be made of many types of solid materials, such as silicon, glass, plastics, and ceramics. The substrate 720 may be porous and may be non-porous. The electrically heating elements 750 may be fabricated by forming thin electrically conductive wires (e.g. having a cross-sectional area of 5 micron by 10 micron for deposited gold as the conductive material). Depending on the specific requirement, heating elements with appropriate resistors may be used with proper choice of materials and structural geometries for the elements. How large the resistors of the heating elements should be depends on the heating requirement for each heating element and depends on the electric currents/voltages that are applied to the heating elements. The heating elements 750 may be fabricated using standard microfabrication and micromachining techniques (e.g. thin conductive film fabrication technologies). Many examples and principles of microlithography and microfabrication are provided in "Handbook of Microlithography, Micromachining and Microfabrication, by Rai-Choudhury P. (Editor), Volume 2: Micromachining and microfabrication. SPIE Optical Engineering Press, Bellingham, Washington, USA (1997)". Based on the specific geometry of the electrical heating elements, those who are skilled in the microfabrication on substrates and in the thin filming material processing may readily choose appropriate materials and methods for fabricating the heating elements 750. Individually addressable heating elements are each connected to the pads 780 on the substrate 720. The pads 780 and connection electrodes between the pads and the heating elements can also be fabricated using the methods similar to those for fabricating the array of heating elements. In Figure

8A, for clarity, we only show the electrical connection between some heating elements to the connection pads although all the elements have corresponding connection pads. The heating elements in the array do not have to be individually addressable. Depending on the specific requirement, some of, or all of the heating elements may be connected in parallel or in series so that they are addressed together. The electric signals from the signal source 790 can be applied to the heating elements 750 through the connection pads 780, the joule heating due to the resistance of the elements 750 will result in an increase in the local temperature.

After the heating elements are fabricated on the substrate 720 in Figure 8, a thin insulating, dielectric layer 460 is further coated on the substrate 720. This layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to several hundred microns. Preferably, the dielectric layer is between 1 micron and 10 micron. Note for clarity, the dielectric layer 460 is shown separated from the substrate 720. The surface of dielectric layer 460 is further processed or polished for smoothness so that an individually addressable electrode array 810 can be fabricated on the dielectric layer 460. Here each electrode element has a hexagon shape (with dimensions from several micron, e.g. side width = 3 micron, to several mm, e.g. side width = 3 mm). Each electrode element can be addressed externally with electrical signals through connection pads 812 shown in Figure 8. For clarity, in Figure 8, we only show the connections between some electrode elements and connection pads. The methods for fabricating such electrode array 810 are similar to those described for the interdigitated electrode array shown in Figure 3A and 3C. Note that after the fabrication of electrode array 810, it is necessary to ensure that the connection pads 780 for the heating elements on the substrate 720 are still open to external connection. This can be achieved by either protecting these pads 780 during the fabrication process, alternatively, can be achieved through proper photolithography procedures.

The heating elements 750 in Figure 8 can be energized by electric signals (current or voltage, and DC or AC) from external signal source 790 through the connections on pad 780. Local temperature change will be resulted in the neighborhood of heating elements. This temperature change will be coupled through the dielectric layer 460 into the medium (not shown in Figure 8) that may be in direct contact or in indirect contact with the dielectric layer 460. The resulting temperature gradient in the medium will cause a motion

in the medium, leading to a velocity field in the medium. The velocity field will exert mechanical forces on the moieties placed or suspended in the medium. The electrode array 810 in Figure 8 may be energized by electric signals (typically DC or low frequency AC from below 1 Hz to several kHz) from signal source 680 through connection pads 812 to produce a DC or low frequency AC electric field in the medium. The electric field will exert electrostatic forces or electrophoretic forces on the moieties in the medium as long as the moieties have net electric charges.

The surface of the dielectric layer 460 that has been processed to support the electrode array 810 as shown in Figure 8 may be further coated or covered with a thin layer of functional materials. In such a case, when the medium is introduced to the apparatus comprising the substrate 720 and the dielectric layer 460, the medium is in indirect contact with the dielectric layer 460. The indirect contact between the medium and the dielectric layer 460 is through the layer of functional materials (and through the electrode array 810 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the dielectric layer 460. Some examples of the functional layer have been provided in the context of describing Figure 3A.

Figure 9 shows schematic drawing of examples of two-force chips of the present invention, capable of producing conventional dielectrophoretic forces and thermal convection-induced mechanical forces. The multiple-force chip in Figure 9 is similar to those in Figure 8, with the differences being in the geometry and structures of electrode array 830 on the substrate 460. Here, the array 830 comprises individually addressable circle electrodes, with the diameters of the electrode elements being between several micron and several mm.

The heating elements 750 in Figure 9 can be energized by electric signals (current or voltage, and DC or AC) from external signal source 790 through the connections on pad 780. Local temperature change will be resulted in the neighborhood of heating elements. This temperature change will be coupled through the dielectric layer 460 into the medium (not shown in Figure 9) that may be in direct contact or in indirect contact with the dielectric layer 460. The resulting temperature gradient in the medium will cause a motion in the medium, leading to a velocity field in the medium. The velocity field will exert mechanical forces on the moieties introduced in the medium. The electrode array 830 in Figure 9 may be energized by electric signals (AC, from below 1 Hz to about 1 GHz) from signal source 380 through connection pads 832 to produce a AC non-uniform electric field

in the medium. The non-uniform electric field will exert conventional DEP forces on the moieties in the medium as long as the moieties have dielectric properties that are different from those of the medium.

Figure 10 shows a schematic drawing of examples of three-force apparatus of the present invention, capable of producing magnetic forces, traveling wave dielectrophoretic forces and optical forces. The apparatus comprises a fluidic chamber, having a two-force chip, a spacer and an optical chip. The two-force chip capable of producing magnetic force and traveling-wave dielectrophoretic forces is fabricated on a substrate 355. The structure and the layout of the two-force chip in Figure 10, and methods for fabricating such a chip are similar to those of the two-force chip shown in Figure 6B. A spacer 850 having appropriate thickness is placed over the two-force chip. The spacer 850 has a channel 852 cut in the center. An optical chip is fabricated on a substrate 870 made of appropriate materials such as glass. An array of optical elements 880 such as lenses, filters is fabricated on the substrate 870. The geometry, the structure and the compositions of the optical elements 880 depend on specific requirements. The optical chip is further processed to have input and output ports 872 and 876. The optical chip, the spacer and the two-force chip are bound together to form one apparatus. These components may be glued together, for example. Note for clarity, these components are shown separated in Figure 10.

In operation, the medium containing the moieties to be manipulated is introduced into the channel 852 of the apparatus through an input port 872. Electrical signals from the signal source 450 can be applied to the electromagnetic units 415 to produce magnetic field that penetrates through the dielectric layer 460 into the medium in the channel 852. The magnetic field can exert magnetic forces on moieties that have certain magnetic properties. Electric signals from the signal source 490 can be applied to the electrode array 480 through connection pads 486 to produce a traveling, non-uniform electric field in the medium. Such electric field can then exert both conventional DEP forces and traveling-wave DEP forces on moieties in the medium. Optical signals 900 from an external optical source (not shown in Figure 10) can be applied to the optical elements 880 on the optical chip so that an optical field is produced in the medium. Such optical field can exert optical radiation forces on the moieties in the medium.

Similar to the multiple-force chips described in Figures 3A-3B, Figure 5A through Figure 9, the surface of the dielectric layer 460 that has been processed to contain the four-

phase linear electrode array can be further covered or coated with a layer of functional material. In such a case, the medium is in indirect contact with the dielectric layer 460. The indirect contact between the medium and the dielectric layer 460 is through the layer of functional materials (and through the electrode array 480 in some regions). The functional material layer may be used for immobilizing certain biomolecules on the surfaces of the dielectric layer 460. Some examples of the functional layer have been provided in the context of Figure 3A.

Figures 11A, 11B and 11C show the schematic drawing of examples of three-force chip of the present invention, capable of producing acoustic forces, magnetic forces, traveling wave dielectrophoretic and/or conventional dielectrophoretic forces.

The chip in Figure 11A comprises a piezoelectric substrate 300 and a substrate 355. Both surfaces 320 and 350 of the piezoelectric substrate 300 are covered with thin conductive layers, which are used as electrodes. The electrodes and the piezoelectric substrate materials are similar to those of piezoelectric substrate in Figure 3B. An array of electromagnetic units 415 is fabricated on a substrate 355. The geometry and structure of the electromagnetic unit 415, the layout of the array, and the methods for fabricating such electromagnetic unit array are similar to those described for electromagnetic unit array shown in Figure 6B. After the array of electromagnetic units 415 is fabricated, the substrate 355 is further covered with a thin insulating, dielectric layer 460. This layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to about 200 microns. Preferably, the dielectric layer is between 1 micron and 10 micron. Note for clarity, the dielectric layer 460 is shown separated from the substrate 355. The surface of dielectric layer 460 is further processed or polished for smoothness so that a four phase, linear, traveling-wave dielectrophoresis array 920 can be fabricated on the dielectric layer 460. The structure of the electrodes 920, the layout of the array, and the methods for fabricating such an array are similar to those described for the electrode array shown in Figure 6B, except that the electrode array is oriented 90 degree from those shown in Figure 6B. Note that after the fabrication of electrode array 920, it is necessary to ensure that the connection pads 440 for the electromagnetic units on the substrate 355 are still open to external connection. This can be achieved by protecting these pads 440 during the fabrication process, or, can be achieved through certain proper photolithography procedures.

Although the substrate 300, the substrate 355 (and the dielectric layer 460 on the substrate) are shown separately in Figure 11A, they are bound together to form one integral chip, just as the two-force chip shown in Figure 3B.

In using the chip in Figure 11A, electrical signals from the signal source 360 can be applied to the piezoelectric substrate 300 to produce acoustic wave fields that is coupled into the medium that is placed or introduced over the surface of the dielectric layer 460. The acoustic field can exert acoustic forces on the moieties in the medium. Electrical signals (i.e. electric currents) from the signal source 450 can be applied to the electric magnetic elements on the substrate 355 to produce magnetic field that penetrates through the dielectric layer 460 into the medium. The magnetic field can exert magnetic forces on moieties that have certain magnetic properties. Electric signals from the signal source 490 can be applied to the electrode array 920 through connection pads 926 to produce a traveling, non-uniform electric field in the medium. Such electric field can then exert both conventional DEP forces and traveling-wave DEP forces on moieties in the medium.

The chip in Figure 11B comprises a piezoelectric substrate 300. Both surfaces of the piezoelectric substrate 300 are covered with thin conductive layers, which are used as electrodes. The electrodes and the piezoelectric substrate materials are similar to those of piezoelectric substrate in Figure 5A. An array of electromagnetic units 415 is fabricated on the substrate 300. The geometry and structure of the electromagnetic unit 415, the layout of the array, and the methods for fabricating such electromagnetic unit array are similar to those described for electromagnetic unit array fabricated on the piezoelectric substrate as shown in Figure 5A. After the array of electromagnetic units 415 is fabricated, the substrate 355 is further covered with a thin insulating, dielectric layer 460. This layer can be made of various materials such as silicon dioxide, or silicon nitrate, or other thin film dielectrics. The layer can have thickness varying from less than 1 micron to about 200 microns. Preferably, the dielectric layer is between 1 micron and 10 micron. Note for clarity, the dielectric layer 460 is shown separated from the substrate 355. The surface of dielectric layer 460 is further processed or polished for smoothness so that an additional traveling-wave dielectrophoresis array 950 can be fabricated on the dielectric layer 460. The electrode array 950 comprises a set of concentric circle-type electrodes, with every fourth circle electrode connected together. The methods for fabricating such an array are similar to those described for the electrode array shown in Figure 6B. For example, multiple conductive layers are necessary for the electrode array 950. If photolithography

technique is used, multiple photomasks are necessary for making the electrode array 950. Note that after the fabrication of electrode array 950, it is necessary to ensure that the connection pads 440 for the electromagnetic units on the substrate 355 are still open to external connection. This can be achieved by either protecting these pads 440 during the fabrication process, alternatively, can be achieved through proper photolithography procedures.

In using the chip shown in Figure 11B, electrical signals from the signal source 360 can be applied to the piezoelectric substrate 300 through electrode pads 435 and bottom surfaces 320 of the substrate 300 to produce acoustic wave fields that is coupled into the medium that is placed or introduced over the surface of the dielectric layer 460. The acoustic field can exert acoustic forces on the moieties in the medium. Electrical signals from the signal source 450 can be applied to the electromagnetic elements 415 to produce magnetic field that penetrates through the dielectric layer 460 into the medium. The magnetic field can exert magnetic forces on moieties that have certain magnetic properties. Electric signals from the signal source 490 can be applied to the electrode array 950 through connection pads 956 to produce a traveling, non-uniform electric field in the medium. Such electric field can then exert both conventional DEP and traveling-wave DEP forces on moieties in the medium.

The chip in Figure 11C comprises a substrate 390 and a substrate 355. An array of piezoelectric elements 395 is fabricated on the substrate 390. The geometry and structure of the piezoelectric elements 395 and the layout of the array are similar to those described for the piezoelectric array as shown in Figure 3C. An array of electromagnetic units 415 and an array of linear, four-phase traveling-wave dielectrophoresis electrodes 960 are co-fabricated on the substrate 355. The geometry and structure of the electromagnetic units 415 and the layout of the electromagnetic array are similar to those described for electromagnetic unit array fabricated on the piezoelectric substrate 300 as shown in Figure 5A. The array of electrodes 960 is connected to every electromagnetic unit, and it involves in multiple, conductive layers. The array of electrodes 960 is used to transport moieties along the direction of the electrode array such as the arrows 962, 965 and 968 indicated in Figure 10. The transportation forces are traveling wave dielectrophoresis forces. Moieties can be switched or controlled at junctions of the electrode branches (such as the junction 966 illustrated in Figure 11C). Although not shown, particle switch structures disclosed in the co-pending USA application, Attorney Docket No. Knobbe, Martens, Olson & Bear,

LLP, ARTLNCO.002a, entitled "APPARATUSES FOR SWITCHING AND MANIPULATING PARTICLES AND METHOD OF USE THEREOF" by Wang et al., filed on October 3, 2000, may be incorporated here. Those who are skilled in microfabrication may readily choose and determine appropriate protocols, methods and processes for co-fabricating the array of electromagnetic unit 415 and the array of electrodes 960.

In using the chip shown in Figure 11C, electrical signals from the signal source 360 can be applied to the array of piezoelectric elements 395 to produce acoustic wave fields in the medium that is placed or introduced over the surface of the substrate 355. The acoustic field can exert acoustic forces on the moieties in the medium. Electrical signals from the signal source 450 can be applied to the electromagnetic elements on the substrate 355 to produce magnetic field in the medium. The magnetic field can exert magnetic forces on moieties that have certain magnetic properties. Electric signals from the signal source 490 can be applied to the electrode array 960 to produce traveling, non-uniform electric fields in the medium. Such electric field can then exert both conventional DEP forces and traveling-wave DEP forces on moieties in the medium. Note that the array of electrodes 960 is used for transporting moieties (such as cells, particles) between different electromagnetic units through traveling-wave DEP forces. Moieties can be transported along the direction of the electrode array such as arrows 962, 965 and 968 indicated in Figure 11C. Moieties can be switched or controlled at junctions of the electrode branches (such as the junction 966 illustrated in Figure 11C).

Similar to the multiple-force chips described above, the surfaces of the three-force chips shown in Figures 11A, 11B and 11C may be further coated with a thin layer of functional materials. The functional material layer may be used for immobilizing certain biomolecules on the chip surfaces. Some examples of the functional layer have been provided in the context of describing Figure 3A.

In the above examples, all the structural elements for producing various types of forces are fabricated on substrates of appropriate materials with various methods such as microfabrication and/or micromachining methods. However, the structural elements can be attached or bond onto the substrates. For example, thin electrical wires may be used as electrodes for generating electric fields. Such wires may be bond to, or attached to the substrate using various methods, such as gluing. In another example, electromagnetic units may be attached to a substrate.

The above examples are included for illustrative purposes only and are not intended to limit the scope of the invention. Many variations to those described above are possible. Examples of these variations include, but not limited to, the substrate materials for making the chips, the electrode structures for generating electric fields, the structure of electromagnetic units for producing magnetic fields, the structures of piezoelectric elements for producing acoustic fields, the structures of optical elements for generating optical fields, the structures of heating/cooling elements for generating temperature gradient, etc. Since modifications and variations to the examples described above will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

Claims

1. A chip for generating physical fields, which chip comprises:

a) a substrate; and

b) at least two different types of built-in structures on said substrate, wherein each of said structures is capable of, in combination with an external energy source, generating one type of physical field.

2. The chip of claim 1, which comprises a plurality of structurally linked substrates.

3. The chip of claim 1, wherein the built-in structures generate at least two different types of physical fields.

4. The chip of claim 1, which comprises two different types of built-in structures that are capable of generating two different types of physical fields.

5. The chip of claim 1, which comprises three different types of built-in structures that are capable of generating three different types of physical fields.

6. The chip of claim 1, which comprises four different types of built-in structures that are capable of generating four different types of physical fields.

7. The chip of claim 1, which comprises more than four different types of built-in structures that are capable of generating more than four different types of physical fields.

8. The chip of claim 1, wherein the built-in structures are in the form of single units.

9. The chip of claim 8, wherein the single units are located in a portion of or in the entire chip.

10. The chip of claim 1, wherein the built-in structures comprise a plurality of microunits.

11. The chip of claim 10, wherein at least some of the microunits are individually addressable.

12. The chip of claim 10, wherein at least some of the microunits are interconnected.

13. The chip of claim 10, further comprising means for selectively energizing any one of the plurality of microunits.

14. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic, optical and velocity fields.

15. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of magnetic, acoustic, optical and velocity fields.

16. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, acoustic, optical and velocity fields.

17. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, optical and velocity fields.

18. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic and velocity fields.

19. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields selected from the group consisting of electric, magnetic, acoustic and optical fields.

20. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of an optical radiation field and a non-uniform AC field.

21. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of a standing-wave acoustic field and an uniform DC electric field.

22. The chip of claim 1, wherein the built-in structures are capable of generating at least two different types of physical fields that do not include the combination of an electric field and a velocity field.

23. The chip of claim 1, wherein at least one built-in structure is capable of generating an electric field.

24. The chip of claim 23, wherein the built-in structure is capable of generating an uniform electrostatic field.

25. The chip of claim 23, wherein the built-in structure is capable of generating a non-uniform AC electric field that has a non-uniform distribution in field magnitude.

26. The chip of claim 23, wherein the built-in structure is capable of generating a non-uniform AC electric field that has a non-uniform distribution in phase values of at least one field component.

27. The chip of claim 23, wherein the built-in structure that generates the electric field comprises at least one microelectrode element.

28. The chip of claim 1, wherein at least one built-in structure is capable of generating a magnetic field.

29. The chip of claim 28, wherein the built-in structure that generates the magnetic field comprises a ferromagnetic material.

30. The chip of claim 28, wherein the built-in structure that generates the magnetic field comprises a microelectromagenetic unit.

31. The chip of claim 1, wherein at least one built-in structure is capable of generating an acoustic field.

32. The chip of claim 31, wherein the built-in structure that generates the acoustic field comprises a piezoelectric material.

33. The chip of claim 1, wherein at least one built-in structure is capable of generating an optical field.

34. The chip of claim 33, wherein the built-in structure that generates the optical field comprises a laser tweezers.

35. The chip of claim 1, wherein at least one built-in structure is capable of generating a velocity field.

36. The chip of claim 35, wherein the velocity field is capable of exerting a mechanical force caused by pressure change in the medium.

37. The chip of claim 36, wherein the built-in structure comprises at least one microfabricated tip/capillary.

38. The chip of claim 35, wherein the velocity field is capable of exerting a mechanical force caused thermal convection.

39. The chip of claim 38, wherein the built-in structure comprises an array of heating and/or cooling units.

40. The chip of claim 1, wherein the built-in structures are micro-scale structures.

41. The chip of claim 40, wherein the micro-scale structures have characteristic dimension of basic structural elements in the range from about 0.1 micron to about 20 mm scale.

42. The chip of claim 1, further comprising a functional layer on the substrate.

43. The chip of claim 1, wherein the substrate comprises a surface that is selected from the group consisting of a silicon, a plastic, a glass, a ceramic, a rubber, and a polymer surface.

44. The chip of claim 43, wherein the silicon surface is a silicon dioxide or a silicon nitride surface.

45. The chip of claim 1, wherein the substrate comprises a surface that is hydrophobic or hydrophilic.

46. A chip for generating physical fields, which chip essentially consists of:

a) a substrate; and

b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination with an external energy source, generating one type of physical field.

47. A chip for generating physical fields, which chip consists of:

a) a substrate; and

b) at least two different types of built-in structures located on said substrate, wherein each of said structures is capable of, in combination with an external energy source, generating one type of physical field.

48. An apparatus for manipulating a moiety, which apparatus comprises:

a) a substrate for holding or supporting a moiety to be manipulated;

b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination with an external energy source, generating one type of physical force on said moiety.

49. The apparatus of claim 48, wherein the internal structures are built-in structures located on said substrate.

50. The apparatus of claim 48, wherein the internal structures are not located on the substrate.

51. The apparatus of claim 48, which comprises a plurality of structurally linked substrates.

52. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces on said moiety.

53. The apparatus of claim 48, which comprises two different types of internal structures that are capable of generating two different types of physical forces on said moiety.

54. The apparatus of claim 48, which comprises three different types of internal structures that are capable of generating three different types of physical forces on said moiety.

55. The apparatus of claim 48, which comprises four different types of internal structures that are capable of generating four different types of physical forces on said moiety.

56. The apparatus of claim 48, which comprises more than four different types of internal structures that are capable of generating more than four different types of physical forces on said moiety.

57. The apparatus of claim 48, wherein the internal structures are in the form of single units that are located in a portion of or in the entire substrate.

58. The apparatus of claim 48, wherein the internal structures comprise a plurality of microunits.

59. The apparatus of claim 58, wherein at least some of the microunits are individually addressable.

60. The apparatus of claim 58, wherein at least some of the microunits are interconnected.

61. The apparatus of claim 58, further comprising means for selectively energizing any one of the plurality of microunits.

62. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force, mechanical force, and optical radiation force.

63. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces that do not include the combination of an optical radiation force and a conventional dielectrophoretic force.

64. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces that do not include the combination of an acoustic force and an electrostatic force.

65. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces that do not include the combination of a mechanical force and an electric force.

66. The apparatus of claim 48, wherein at least one internal structure is capable of generating an electric force.

67. The apparatus of claim 66, wherein the internal structure is capable of generating an electrostatic force on charged particles.

68. The apparatus of claim 66, wherein the internal structure is capable of generating a conventional dielectrophoretic force.

69. The apparatus of claim 66, wherein the internal structure is capable of generating a traveling-wave dielectrophoretic force.

70. The apparatus of claim 66, wherein the internal structure that generates electric force comprises at least one microelectrode element.

71. The apparatus of claim 48, wherein at least one internal structure is capable of generating a magnetic force.

72. The apparatus of claim 71, wherein the internal structure that generates a magnetic field comprises a ferromagnetic material.

73. The apparatus of claim 71, wherein the internal structure that generates a magnetic field comprises a microelectromagenetic unit.

74. The apparatus of claim 48, wherein at least one internal structure is capable of generating an acoustic force.

75. The apparatus of claim 74, wherein the internal structure that generates an acoustic force comprises a piezoelectric material.

76. The apparatus of claim 48, wherein at least one internal structure is capable of generating a mechanical force caused by pressure change in the medium.

77. The apparatus of claim 76, wherein the internal structure that generates a mechanical force comprise at least one microfabricated tip/capillary.

78. The apparatus of claim 48, wherein at least one internal structure is capable of generating an optical radiation force.

79. The apparatus of claim 78, wherein the internal structure that generates the optical radiation force comprises a laser tweezers.

80. The apparatus of claim 48, wherein at least one internal structure is capable of generating a mechanical force caused thermal convection.

81. The apparatus of claim 80, wherein the internal structure that generates a thermal gradient force comprises an array of heating and/or cooling units.

82. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of magnetic force, acoustic force, mechanical force and optical radiation force.

83. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, acoustic force, mechanical force and optical radiation force.

84. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, mechanical force and optical radiation force.

85. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force and optical radiation force.

86. The apparatus of claim 48, wherein the internal structures are capable of generating at least two different types of physical forces selected from the group consisting of electrical force, magnetic force, acoustic force and mechanical force.

87. The apparatus of claim 48, wherein the internal structures are micro-scale structures.

88. The apparatus of claim 87, wherein the micro-scale structures have characteristic dimension of basic structural elements in the range from about 0.1 micron to about 20 mm scale.

89. The apparatus of claim 48, further comprising a functional layer on the substrate.

90. The apparatus of claim 48, wherein the substrate comprises a surface that is selected from the group consisting of a silicon, a plastic, a glass, a ceramic, a rubber, and a polymer surface.

91. The apparatus of claim 90, wherein the silicon surface is a silicon dioxide or a silicon nitride surface.

92. The apparatus of claim 48, wherein the substrate comprises a surface that is hydrophobic or hydrophilic.

93. The apparatus of claim 48, wherein the apparatus comprises a fluidic chamber that comprises a substrate and a housing, said fluidic chamber is capable of holding or supporting or containing a moiety to be manipulated.

94. The apparatus of claim 93, wherein the fluidic chamber is a closed one and comprises at least one inlet port and at least one outlet port.

95. The apparatus of claim 48, which does not contain a monitoring or detecting device.

96. An apparatus for manipulating a moiety, which apparatus essentially consists of:

a) a substrate for holding or supporting a moiety to be manipulated;

b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination with an external energy source, generating one type of physical force on said moiety.

97. An apparatus for manipulating a moiety, which apparatus consists of:

a) a substrate for holding or supporting a moiety to be manipulated;

b) at least two different types of structures internal to said apparatus, wherein each of said internal structures is capable of, in combination with an external energy source, generating one type of physical force on said moiety.

98. A combination, which combination comprises:

a) at least two apparatuses of claim 48; and

b) means for transporting a moiety to be manipulated between said apparatuses.

99. An apparatus, which apparatus comprises the chip of claim 1.

100. A method for manipulating a moiety, which method comprises:

a) introducing a moiety to be manipulated into the apparatus of claim 48; and

b) allowing the internal structures of the apparatus, in combination with external energy sources, to exert at least two different types of physical forces on said moiety,

whereby said moiety is manipulated by said physical forces.

101. The method of claim 100, wherein the at last two different types of physical forces are exerted on the moiety sequentially.

102. The method of claim 100, wherein the at last two different types of physical forces are exerted on the moiety simultaneously.

103. The method of claim 100, wherein a plurality of moieties are manipulated simultaneously.

104. The method of claim 103, wherein the plurality of moieties are manipulated simultaneously through use of more than one type of physical forces so that at least two moieties are manipulated by different types of physical forces.

105. The method of claim 100, wherein a plurality of moieties are manipulated sequentially.

106. The method of claim 105, wherein the plurality of moieties are manipulated sequentially through use of more than one type of physical forces so that at least two moieties are manipulated by different types of physical forces.

107. The method of claim 100, wherein the moiety to be manipulated is contained in a mixture and the moiety is selectively manipulated.

108. The method of claim 100, wherein the moiety to be manipulated constitutes a mixture and the entire mixture is manipulated.

109. The method of claim 100, wherein the moiety to be manipulated is selected from the group consisting of a cell, a cellular organelle, a virus, a molecule and an aggregate or complex thereof.

110. The method of claim 109, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a fungus cell, a bacterium cell, a recombinant cell and a cultured cell.

111. The method of claim 109, wherein the cellular organelle is selected from the group consisting of a nuclei, a mitochondrion, a chloroplast, a ribosome, an ER, a Golgi apparatus, a lysosome, a proteasome, a secretory vesicle, a vacuole and a microsome.

112. The method of claim 109, wherein the molecule is selected from the group consisting of an inorganic molecule, an organic molecule and a complex thereof.

113. The method of claim 112, wherein the inorganic molecule is an ion selected from the group consisting of a sodium, a potassium, a magnesium, a calcium, a chlorine, an iron, a copper, a zinc, a manganese, a cobalt, an iodine, a molybdenum, a vanadium, a nickel, a chromium, a fluorine, a silicon, a tin, a boron and an arsenic ion.

114. The method of claim 112, wherein the organic molecule is selected from the group consisting of an amino acid, a peptide, a protein, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a vitamin, a monosaccharide, an oligosaccharide, a carbohydrate, a lipid and a complex thereof.

115. The method of claim 100, which is capable of transporting, focusing, enriching, concentrating, aggregating, trapping, repulsing, levitating, separating, fractionating, isolating or directing linear or other directed motion of the moiety.

116. A method for manipulating a moiety, which method comprises exerting at least two different types of physical forces on a moiety, whereby said moiety is manipulated by said physical forces.

117. The method of claim 116, wherein the moiety is manipulated in a liquid medium.

118. The method of claim 117, wherein the moiety is manipulated in a liquid container selected from the group consisting of a beaker, a flask, a cylinder, a test tube, an enpindorf tube, a centrifugation tube, a culture dish and a multiwell plate.

Figure 1

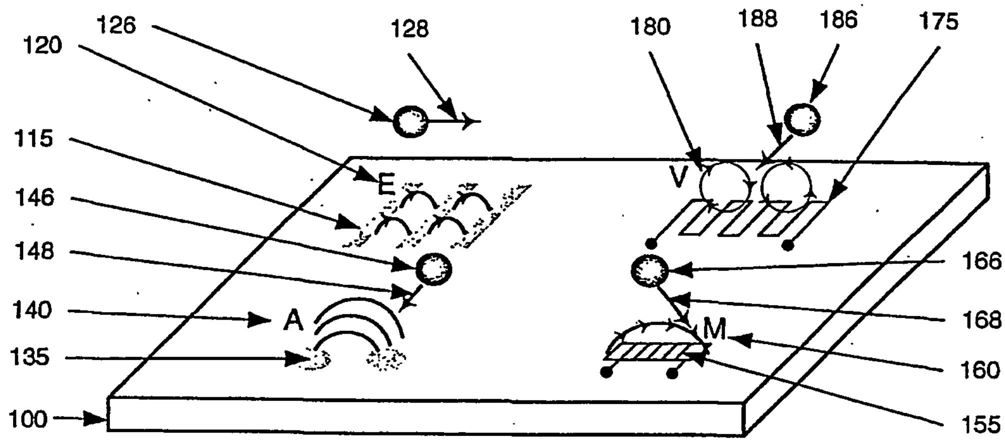

Figure 2

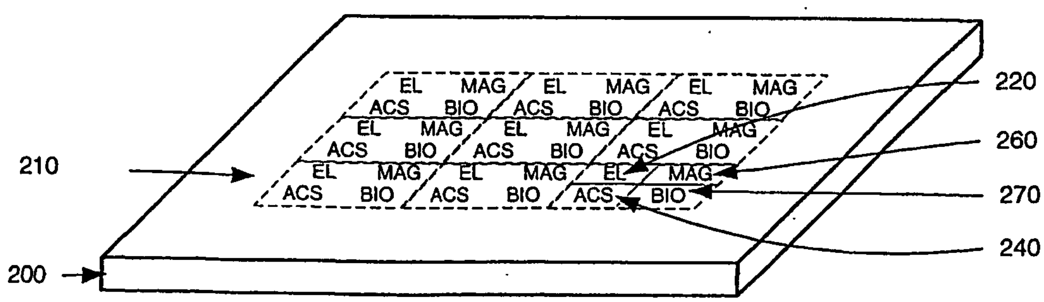

Figure 3A

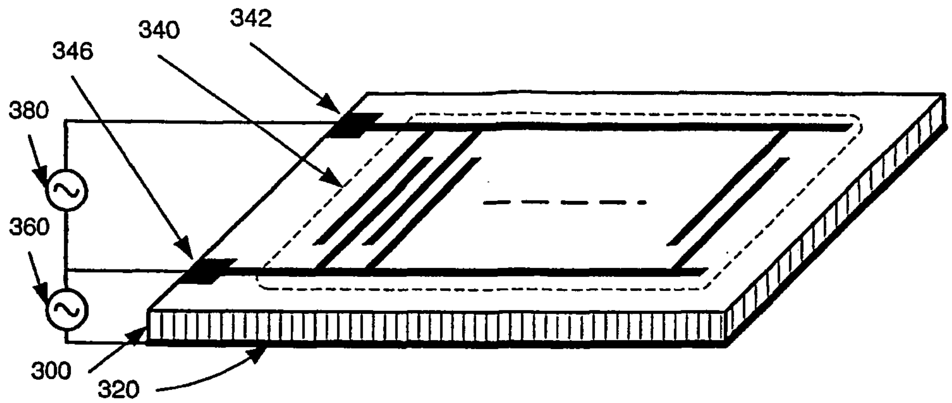

Figure 3B

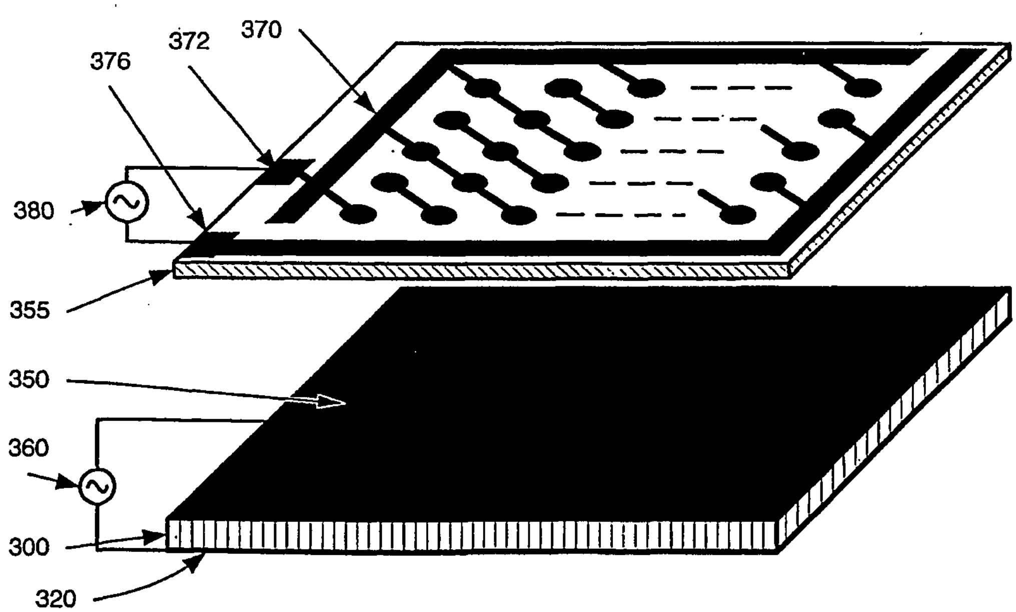

Figure 3C

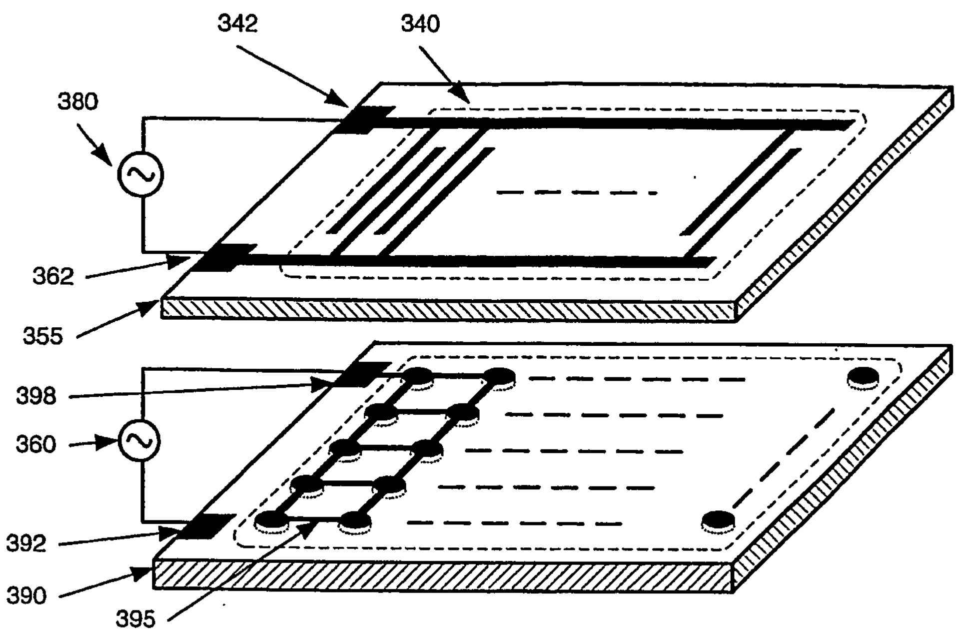

Figure 4

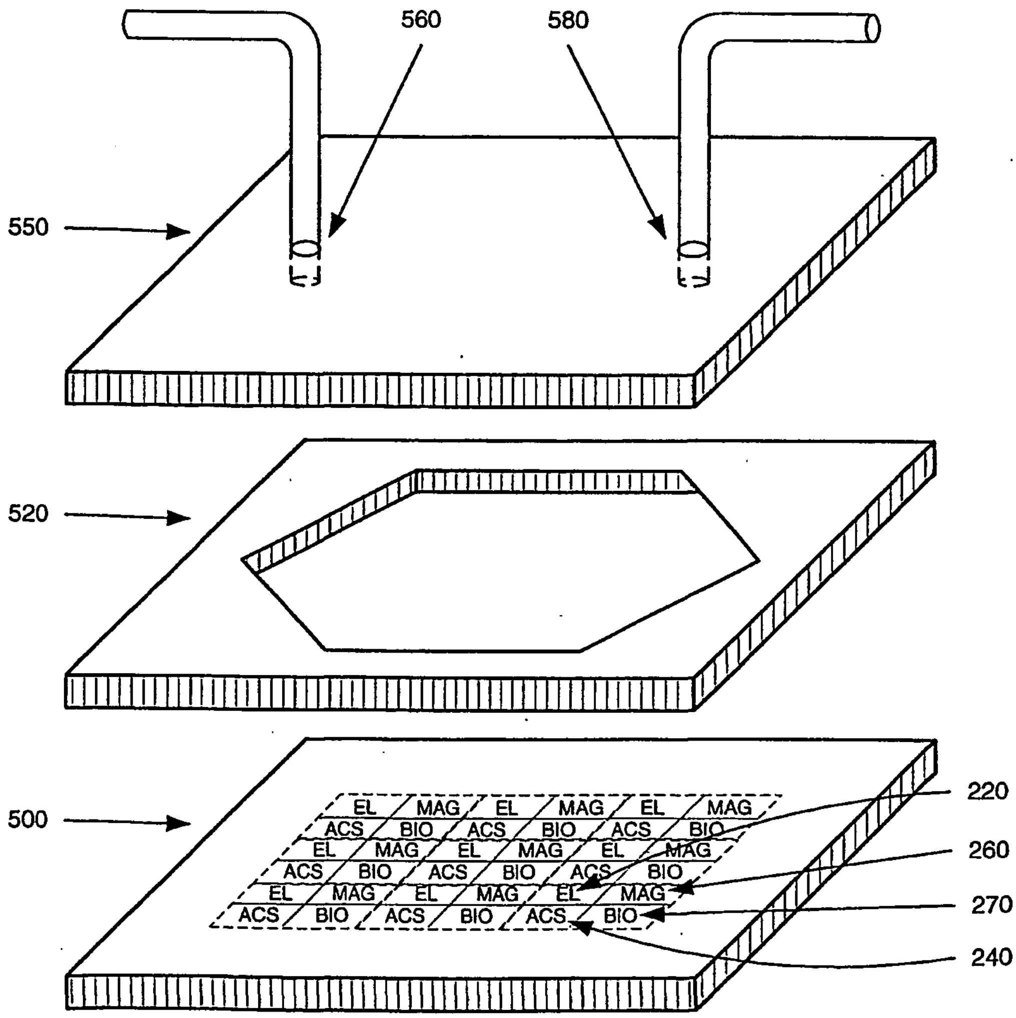

Figure 5A

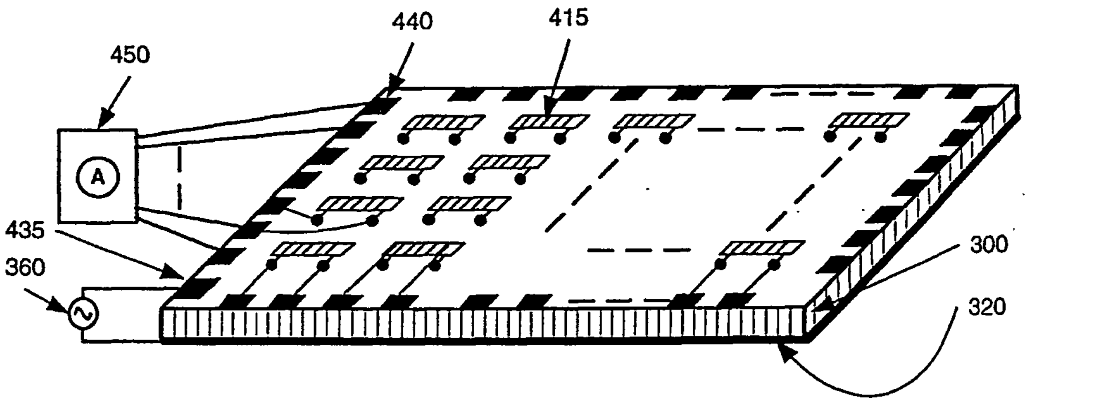

Figure 5B

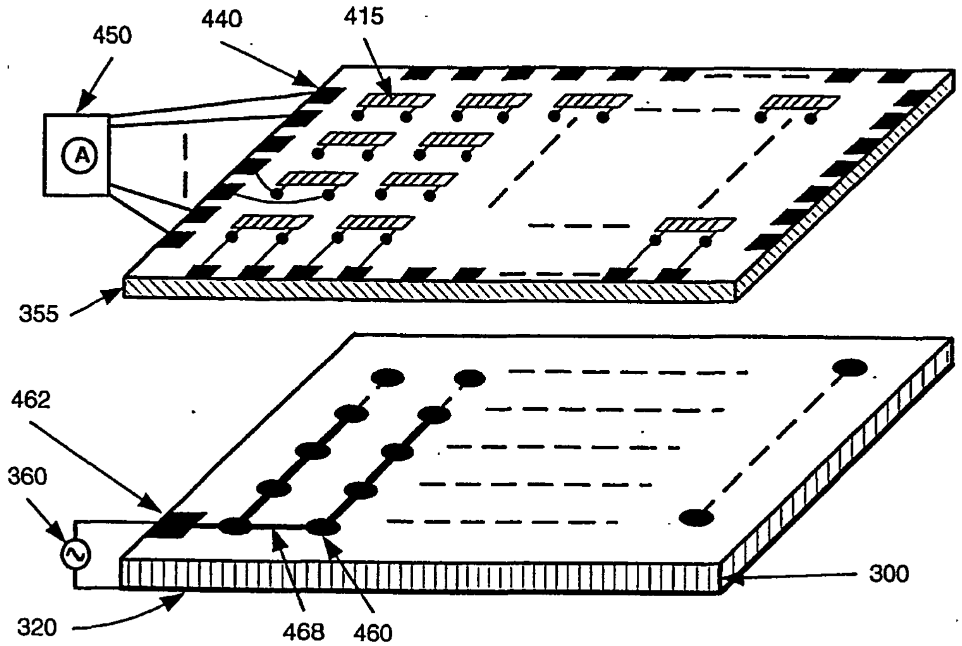

Figure 5C

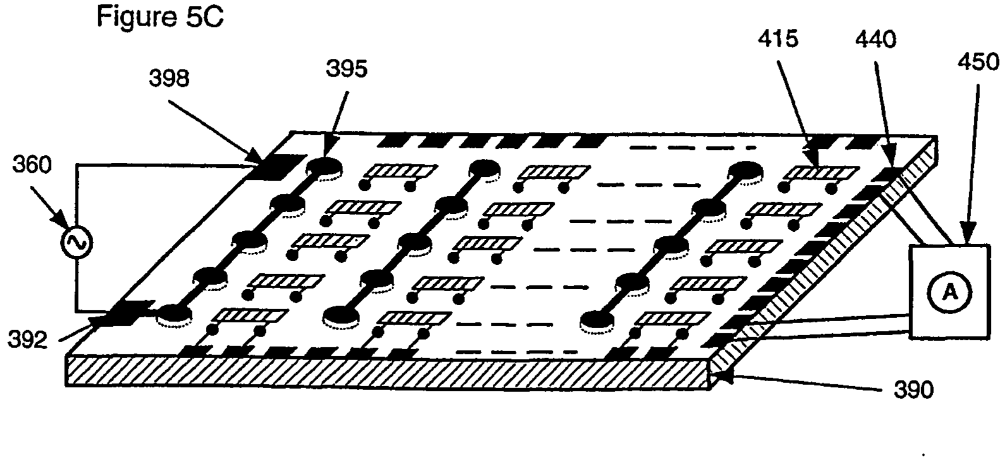

Figure 6A

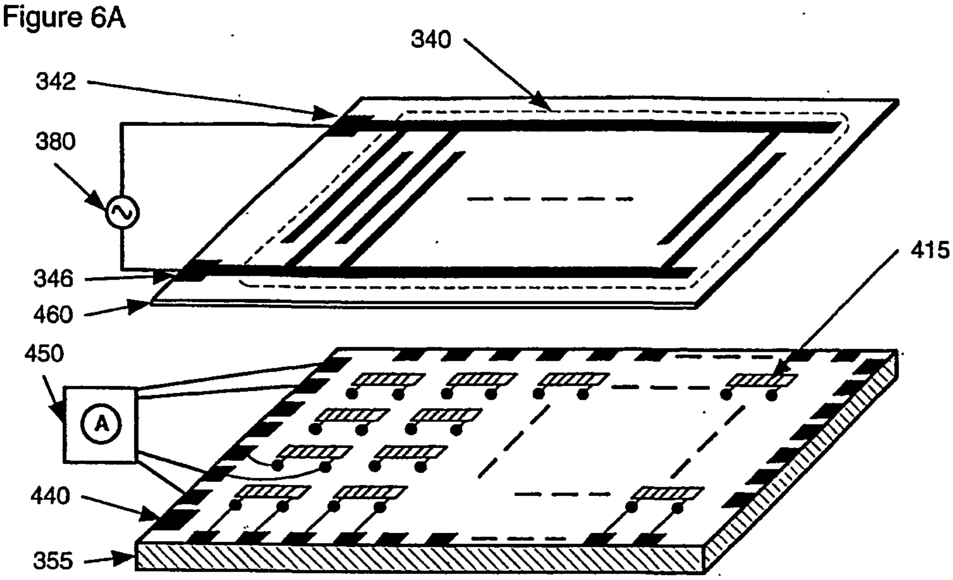

Figure 6B

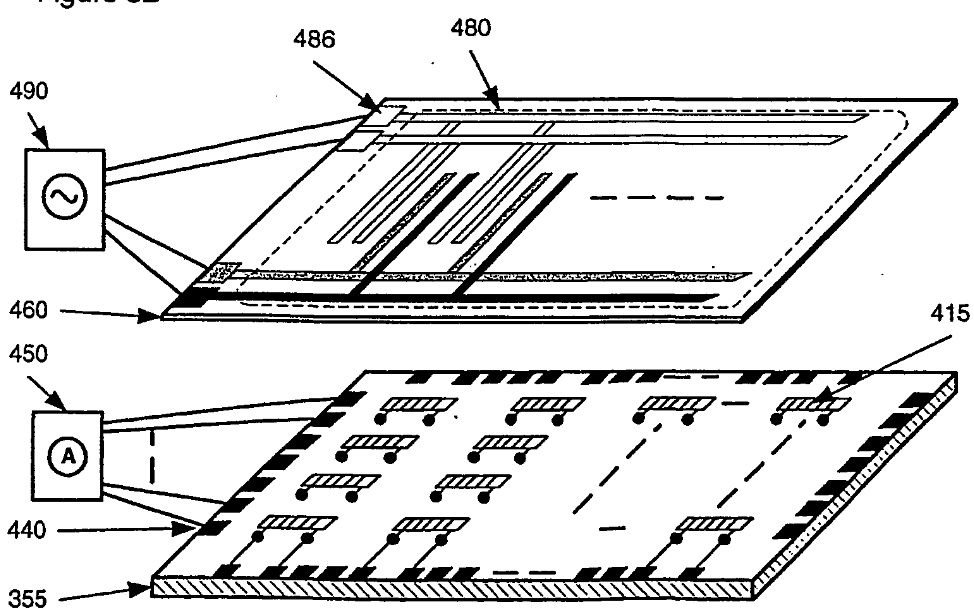

Figure 6C

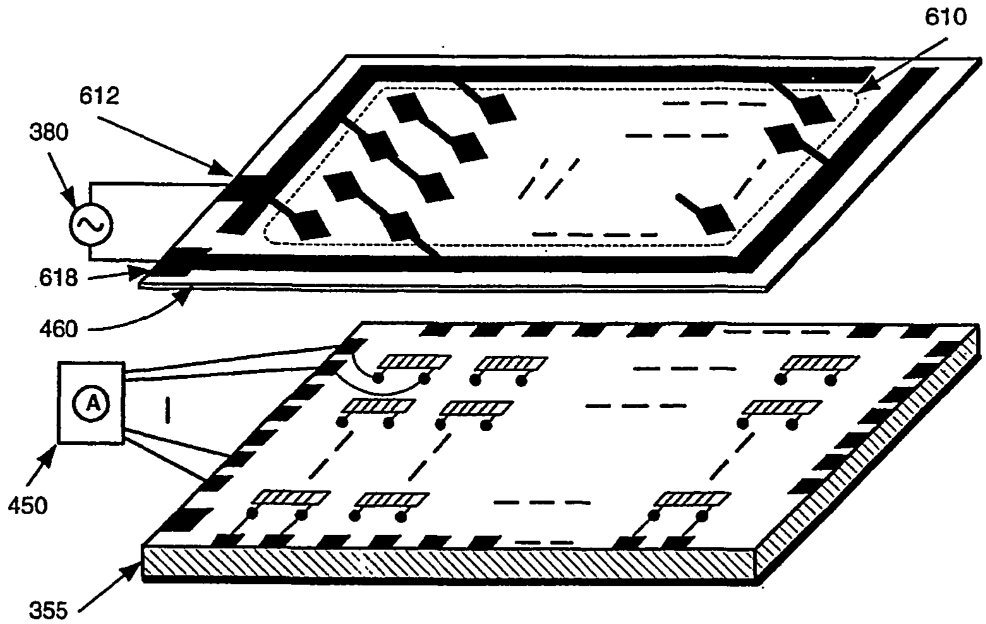

Figure 6D

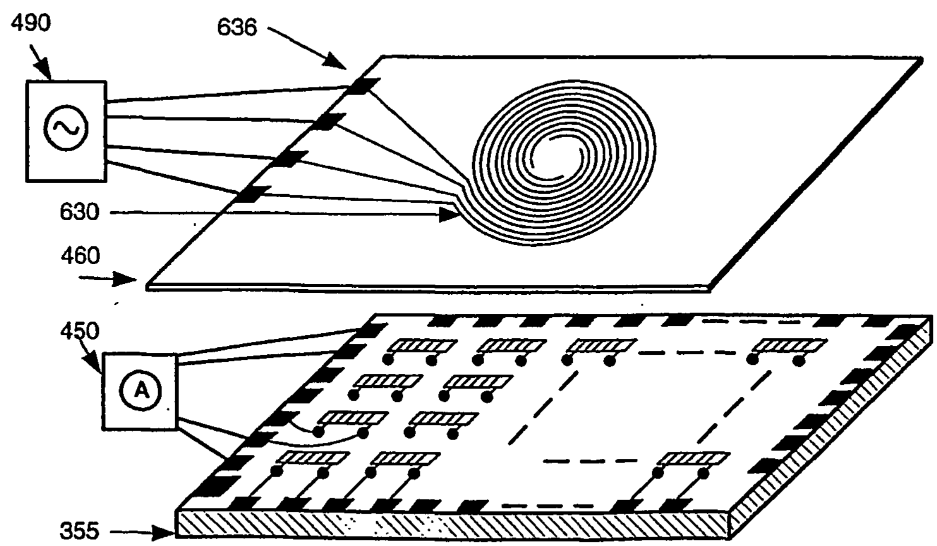

Figure 7A

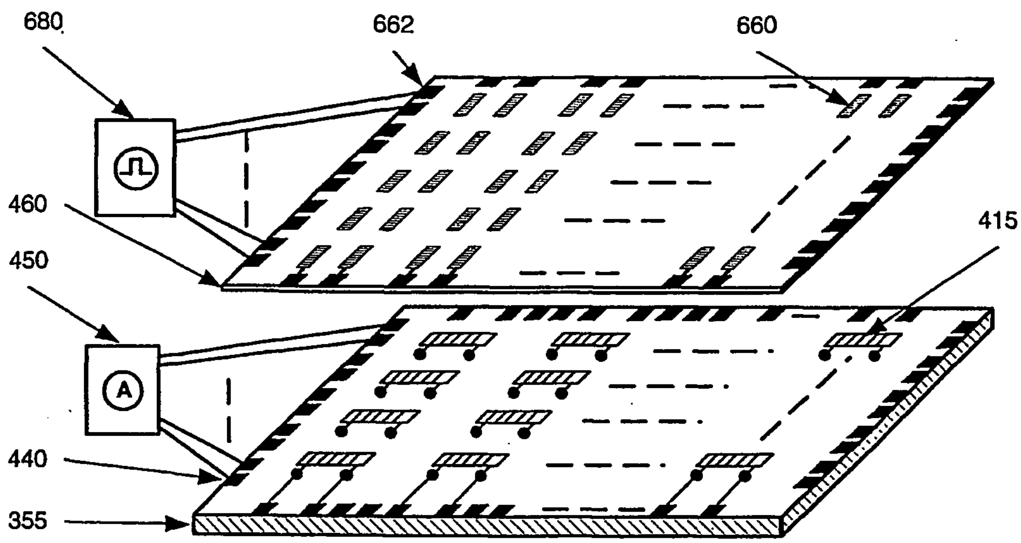

Figure 7B

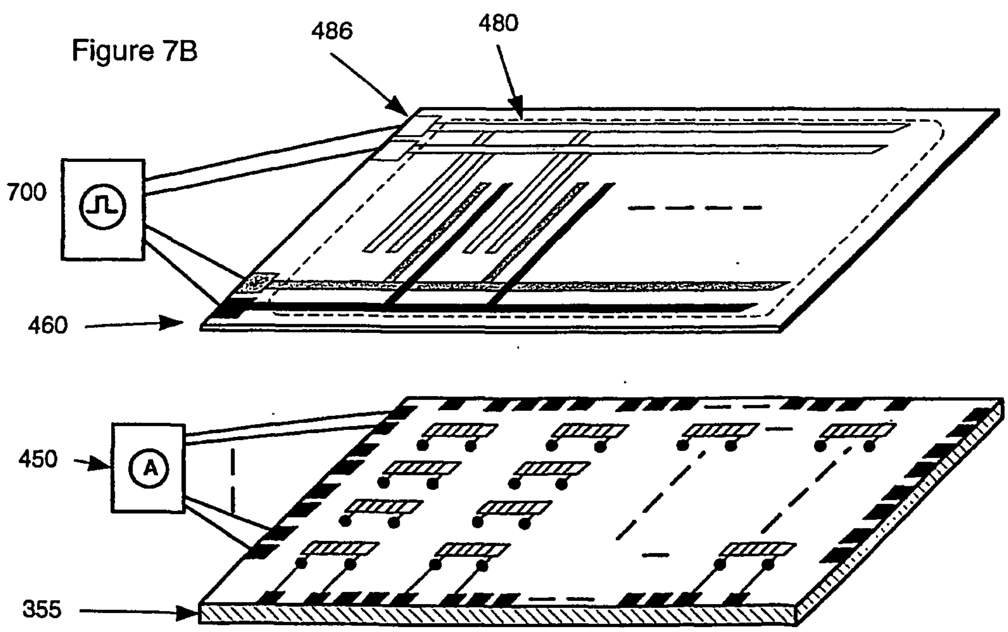

Figure 8

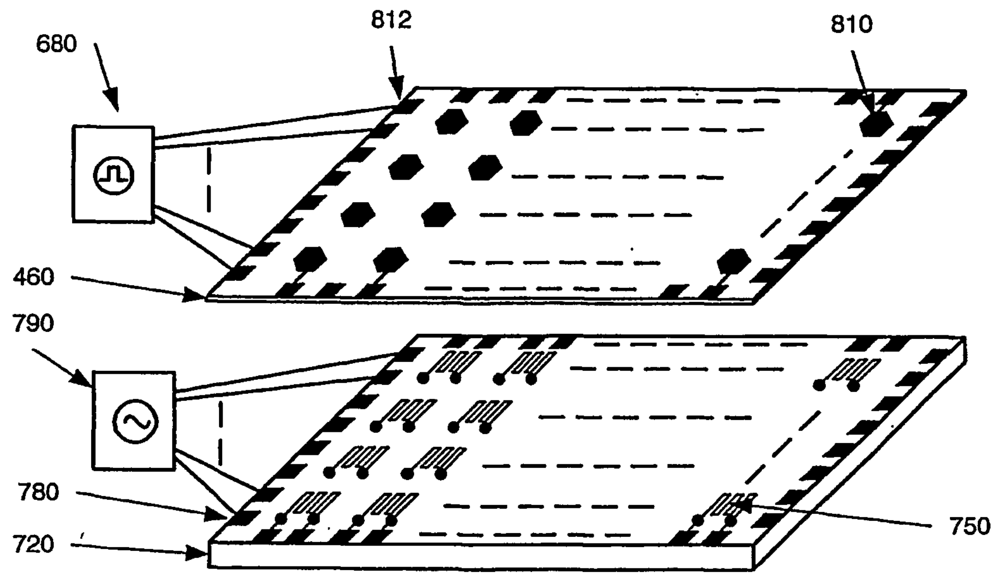

Figure 9

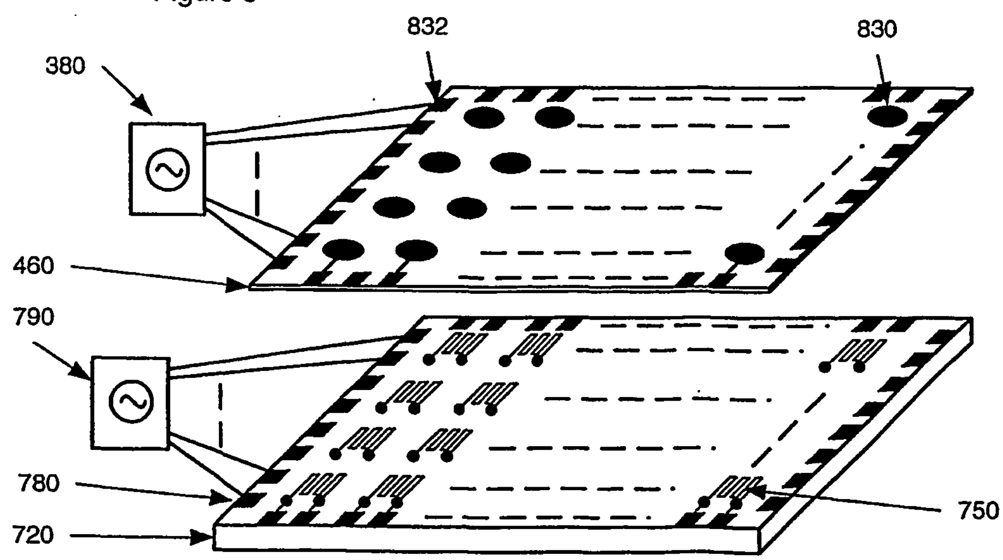

Figure 10

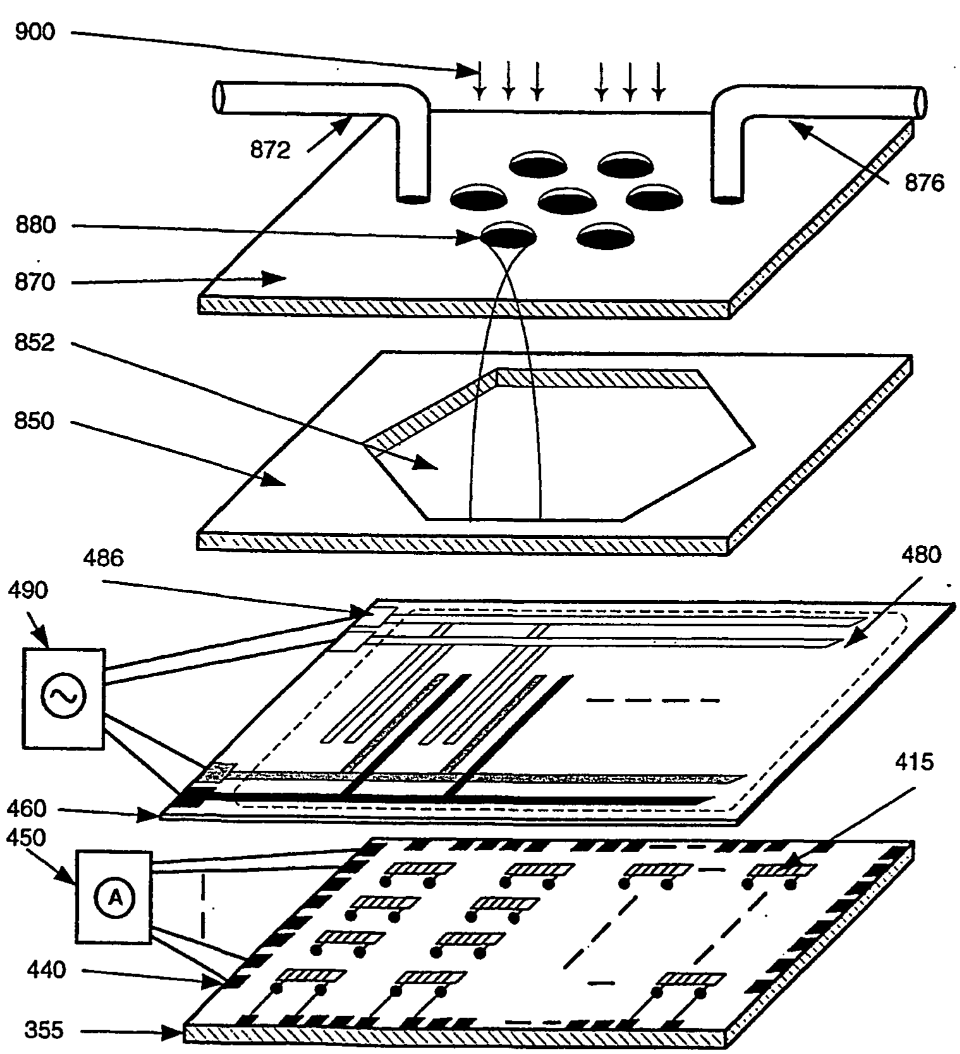

Figure 11A

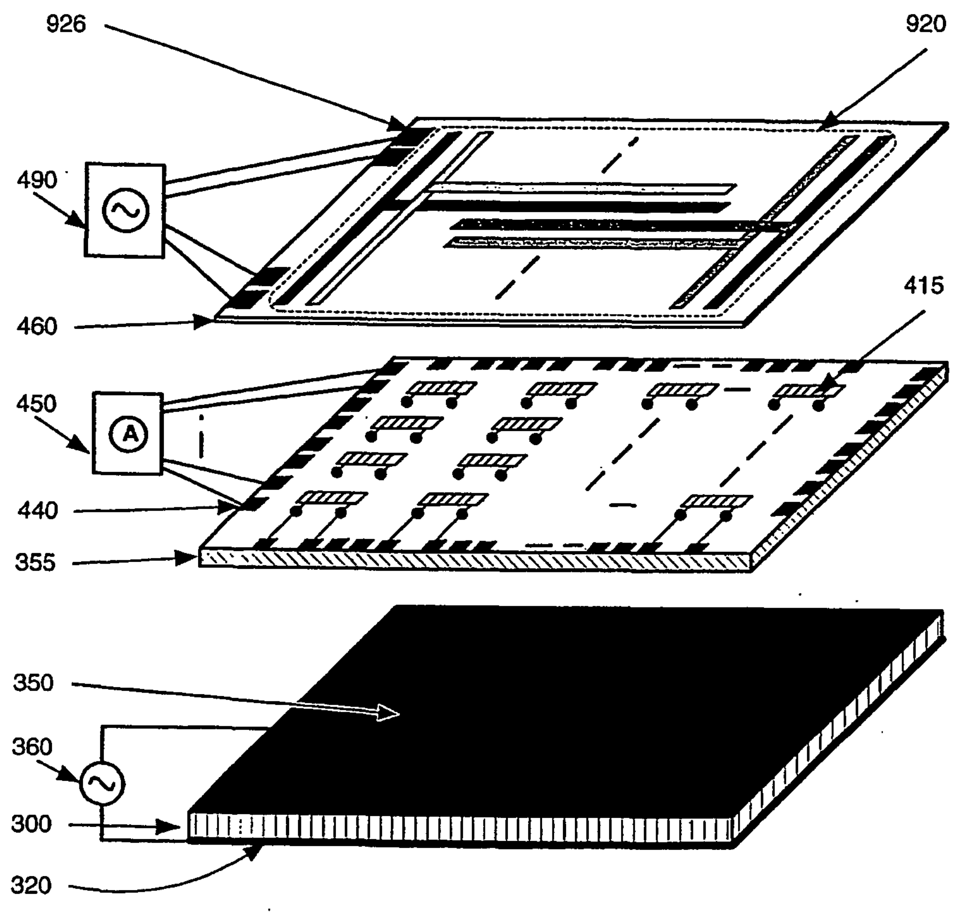

Figure 11B

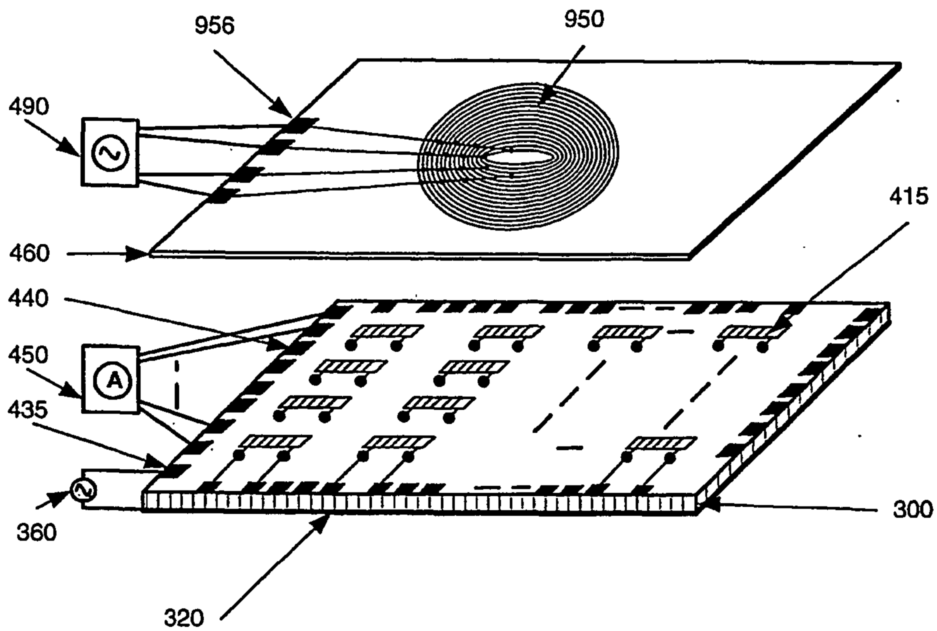

Figure 11C